

Reintroduction ecology of the Eurasian crane *Grus grus*

Submitted by

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To my parents

Abstract

Successful conservation strategies to increase the survival prospects of a population must be accompanied by an accurate knowledge of the population dynamics. In this thesis, I examine the population dynamics of Eurasian cranes (*Grus grus*) in the UK and assess the impact of a reintroduction on the future population size and distribution. Additionally, I cover other aspects of crane conservation, such as public engagement in crane conservation and crane resilience to extreme weather events. To do so, I use a wide range of tools: Internet-based monitoring methods, stochastic matrix population models, point process models and GPS-accelerometer tracking devices. I show that there is a geographic gradient in interest in reintroduction projects; people living near the project area have a greater interest than people from areas further away. I also show that the UK crane population is acting as a pseudo-sink; current levels of survival and productivity allow the population's persistence but immigration is driving population recovery. Nevertheless, the productivity of the UK population is low and measures to improve it should be implemented. The reintroduction of 90 birds in the Somerset Levels has decreased the relative importance of immigration and is likely to increase the projected population size by 50% over the next 50 years. However, the increase in population numbers will not be accompanied by a large expansion in the population range. Cranes will likely colonize large wetlands with low perimeter-to-area ratios near already occupied areas, but the reintroduction will increase the dispersal potential of the species. Finally, I find that during an extreme flooding event, crane foraging areas became limited to a small unflooded patch, but cranes coped with this event through behavioural flexibility, by increasing their foraging time. Together, these results highlight the importance of reaching a broad understanding of population dynamics in order to implement effective conservation strategies.

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Chapter 1

General introduction

1.1 General framework

Scientific evidence highlights that we might be entering the sixth mass extinction, with extinction rates 100-1,000 times higher than the geological background rates (Pimm *et al.* 1995; Barnosky *et al.* 2011) and a quarter of all vertebrate species predicted to be lost in the next century (Baillie *et al.* 2010). The rate of population destructions is extraordinary: over 16 million plant and animal populations are disappearing annually in tropical forests alone, 338 vertebrate species have become extinct since 1500 and we have already lost between a fifth and a quarter of pre-agricultural bird numbers (Hughes, Daily & Ehrlich 1997; Gaston, Blackburn & Goldewijk 2003; Ceballos *et al.* 2015). But, despite international efforts to slow the rate of biodiversity loss, extinction rates are predicted to increase in the following years (Butchart *et al.* 2010).

In this scenario, where many species or populations are already locally extirpated, traditional measures for biodiversity conservation such as creation and management of protected areas and biological corridors are not sufficient to counter the loss of biodiversity, and alternative forms of management must be implemented (IUCN 2013; Seddon *et al.* 2014). Conservation translocations have been suggested as a useful management technique to, among other things, increase species abundance, to speed up population growth, to reduce the risks of genetic or demographic collapse due to stochastic effects and inbreeding depression or to overcome barriers to natural dispersal (Ewen 2012; Deinet *et al.* 2013; IUCN 2013). However, until recently, a theoretical framework on conservation translocations was lacking (Ewen 2012).

Conservation translocations refer to deliberate movements of organisms from one site to another to be released (IUCN 2013). There are two different types of

action depending on the historical range of the species (IUCN 2013; Seddon *et al.* 2014). If translocations take place within the species indigenous range they are considered reintroductions or reinforcements, depending whether the species is locally extirpated or is still present in the area respectively (IUCN 2013; Seddon *et al.* 2014). However, more recently, conservation introductions - translocations outside the historical range - are starting to take place due to human-induced habitat modification and climate change (Seddon *et al.* 2014). There are two types of conservation introduction, depending on their goal: first, assisted colonisations, which intend to prevent the extinction of taxa by moving individuals to new areas where conditions are suitable; second, ecological replacements, which intend to restore an ecological function in an ecosystem (Seddon *et al.* 2014). Usually, the introduced individuals substitute the function of a species that is already extinct (Seddon *et al.* 2014).

Introducing animals to a new environment is problematic and assessing the global degree of success of reintroductions is difficult since researchers tend to publish only positive results, thus rates of success tend to be overestimated (Seddon, Armstrong & Maloney 2007; Sutherland *et al.* 2010). A review of 180 reintroductions projects from 1980 to 2000 found that 27% of the reintroductions failed while the success of 47% was uncertain (Fischer & Lindenmayer 2000). Nevertheless, in recent years the rate of success has increased considerably; several reports published by the IUCN/SSC Re-introduction Specialist Group indicate that the rate of failure is less than 10% (Soorae 2008; Soorae 2011; Soorae 2013). This fact could be due to the increase in reintroduction research; the number of reintroduction-related papers published in peer-reviewed journals increased remarkably from 2000 onwards (Fig 1.1). While in the mid-90s less than 10 papers were published annually, in 2005 more than 70 studies were

produced (Seddon, Armstrong & Maloney 2007). This has led to a better understanding of reintroduction difficulties and a new and recognisable field of research, reintroduction biology (Seddon, Armstrong & Maloney 2007; Ewen 2012).

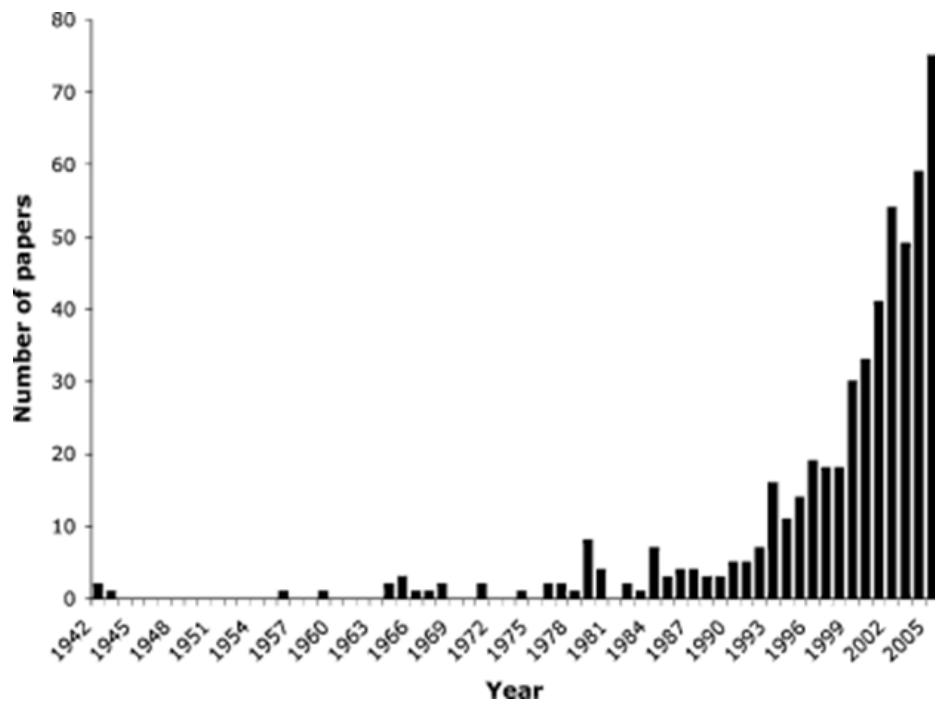


Fig 1.1: Number of reintroduction-related papers published in peer-reviewed journals by year since the first records located up to 2005. Source: Seddon, Armstrong & Maloney 2007.

Under this framework several integrated approaches to address reintroduction targets have been developed, such as a set of minimum standards to monitor reintroduction projects. According to Sutherland *et al.* 2010, to be able to learn from the experience each reintroduction project should (1) document the release before it takes place; (2) consider the monitoring objectives; (3) publish the plan; (4) carry out a post-release monitoring, documenting the size of the population, and the sex and age class of the individuals and (5) document the results. Other approaches suggest addressing key aspects of reintroductions at different levels (Armstrong & Seddon 2008). At a population level, the following

should be assessed: (1) the optimal size and composition of the released group; (2) the management strategies affecting survival and dispersion; (3) the habitat conditions needed for population persistence and (4) the role played by genetics. At a metapopulation level (i.e. considering the source the recipient population and the populations that could become interconnected by the translocation) the questions to address would be (5) the impact on the source population; (6) the optimal allocation of individuals among sites, in the case of a multi-site reintroduction and (7) if translocation should be used to compensate for isolation. Finally, at ecosystem level the key questions would be (8) whether the target species and its parasites are native to the ecosystem; (9) whether the ecosystem will be affected by the target species and its parasites and (10) whether the order of reintroductions will affect the ultimate species composition, in the case of multiple reintroductions. Hence, reintroductions require complex, intense and expensive planning and monitoring strategies, which could explain the difficulties in implementing them successfully. Nevertheless, there are some success stories that exemplify the potential of translocations to bring species and populations back from the brink of extinction.

In New Zealand, for example, the population of Kakapo (*Strigops habroptilus*, a large flightless nocturnal parrot) reached a minimum of 50 individuals in 1995 due to several issues: the introduction of exotic predators, low genetic diversity, infrequent breeding attempts and poor chick survival. To overcome this challenging situation, a number of measures were implemented. The key action was to translocate birds to predator-free islands with suitable habitats. Productivity was boosted by implementing supplementary feeding. Finally, to maximize genetic diversity, semen for artificial insemination was collected and mate access was regulated. This set of measures was successful, such that by

2011 the population comprised 122 individuals (see detailed description in Jones & Merton 2012).

Another classic example of a successful reintroduction project with cascading consequences in the ecosystem was the reintroduction of 31 wolves (*Canis lupus*) into Yellowstone National Park (USA) in the winters of 1995/1996, after they were extirpated from the park in the 1920s (Bangs & Fritts 1996). Currently there are at least 104 wolves in 11 packs living primarily in Yellowstone National Park (Smith *et al.* 2015). During the seventy-year period of wolf absence, the elk (*Cervus elaphus*) population increased to >15,000 individuals due to the lack of top-down regulation, which led to an increase in herbivore pressure on the vegetation communities and some plant species (aspen *Populus tremuloides*, cottonwoods *Populus spp.*, and willows *Salix spp.*) declined significantly (Ripple *et al.* 2001; Ripple & Beschta 2012). The reintroduction of wolves in the area reduced the elk population, which subsequently reduced browsing pressure and allowed the plant community to recover (Ripple *et al.* 2001; Ripple & Beschta 2012). Moreover, an increase in the number of beavers (*Caster canadensis*) and bison (*Bison bison*) has also been reported, possibly linked to the higher availability of woody plants and herbaceous resources, and passerine bird species richness increased due to the resurgence of willow trees (Baril 2009; Ripple & Beschta 2012).

Those examples perfectly illustrate the role of conservation translocation in restoring the populations of threatened species and re-establishing ecological processes. Moreover, the recovery of emblematic species, such as kakapos and wolves, is a powerful tool to engage people in conservation. Flagship species can serve as symbols and rallying points to stimulate conservation

awareness and action on the basis of their possession of traits that appeal to the public (Bowen-Jones & Entwistle 2002; Veríssimo *et al.* 2014).

To date, there is still a debate about the attributes of a flagship species. While some authors suggest that flagship species can be used purely as strategic tools to raise public awareness and financial support for conservation, without needing to have a major ecological role (Walpole & Leader-Williams 2002), others consider that flagship species should act as an umbrella for the benefit of the other species in the habitat (Caro & O'Doherty 1999; Roberge & Angelstam 2004).

The most common qualities in flagship species are aesthetic beauty, small population size, limited distribution range and visibility (Ranjith & Tisdell 2005; Veríssimo *et al.* 2014). Those attributes make the study of such species particularly challenging due to small sample size and the necessity to avoid any potential impact on their populations (Cooke 2008). Nevertheless, the exact same reasons make the study of these species necessary. Flagship species appeal to the popular imagination and their extinction would go beyond an ecological tragedy and would affect society, since their disappearance would symbolize the human impact on the environment (Durbin *et al.* 1996).

1.2 Study species

Eurasian cranes (*Grus grus*, cranes hereafter) are large and iconic birds that present a long life expectancy, delayed maturity and low productivity rates, they can rear up two fledglings per year (Archibald & Meine 1996). They occur across Europe, Asia and some parts of Africa (Fig 1.2, BirdLife-International

2017). They have an extremely large breeding range, from Scandinavia and NE Europe to N China and Russian Far East; as well as areas in Turkey, Caucasus, Armenia and possibly Azerbaijan (Fig 1.2, BirdLife-International 2017). However, the species was extirpated from part of its historical breeding range in Europe due mainly to overexploitation and habitat loss. For example, the last breeding cranes in Italy and Austria date from the 19th century and in Hungary and Greece from the 20th century (Archibald & Meine 1996). The species disappeared from the United Kingdom in the 16th century; however, it started recolonizing the country at the end of the 1970s (Stanbury 2011). Cranes are mainly migratory but there are some resident populations in the United Kingdom and Turkey. Migratory cranes spend the winter in France, Iberian Peninsula, N Africa, Middle East, Pakistan, India and China (Fig 1.2, BirdLife-International 2017). Their global population is around 360,000-370,000 individuals; although the global population trend is positive, some populations are decreasing (Delany, Scott & Helmink 2006; BirdLife-International 2015).

The main threats to the species are habitat loss and degradation in both the breeding and wintering areas, change in agricultural land use toward more intensive agriculture and expansion of irrigation systems, nest disturbance and collision with powerlines (Meine & Archibald 1996). Egg collection and hunting are still a concern in some areas in Turkey, Afghanistan and Pakistan (Meine & Archibald 1996). Nevertheless cranes are categorized as *Least Concern* by the International Union for Conservation of Nature (IUCN) (BirdLife-International 2015).

Cranes use wet areas throughout the year. During the breeding season, cranes use a wide variety of shallow wetlands to nest and roost, such as swamps, sedge meadows, moors and bogs (Archibald & Meine 1996; BirdLife-International 2015). However, they frequently use arable fields and grasslands around those areas to forage. During the non-breeding period, they continue to use wet areas to roost (Archibald & Meine 1996). Cranes have a very diverse omnivorous diet during both the breeding and the non-breeding season. They feed on a wide range of plant items, such as grass roots and shoots, rhizomes, tubers, leaves, berries and seeds (Archibald & Meine 1996). They also feed on adult and larval arthropods (eg. beetles, flies, spiders and millipedes), earthworms, snails, frogs, lizards, snakes, small mammals, fishes and sporadically eggs and chicks of small birds (Archibald & Meine 1996).

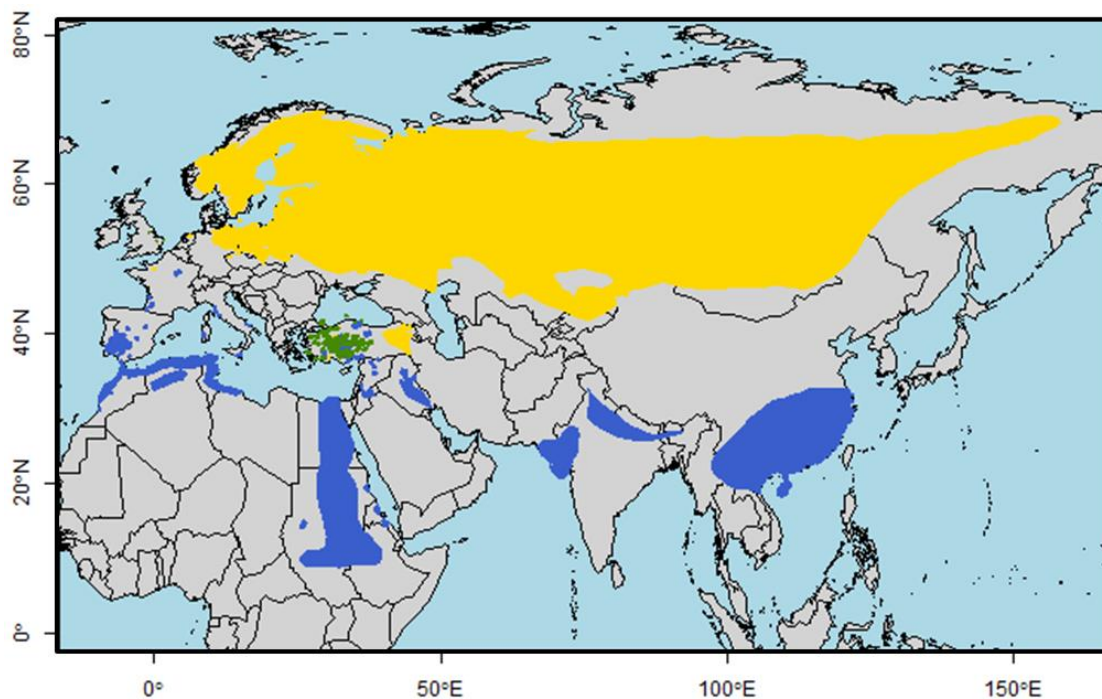


Fig 1.2: Eurasian crane distribution; orange represent the breeding areas, dark blue the wintering areas and green the areas where the species is resident. Source: BirdLife-International 2017.

1.3 Study site

In the United Kingdom (UK), cranes were a common feature on the landscape but hunting and egg harvesting depleted the population until its local extirpation in the 16th century (Gurney 1921; Stanbury 2011). Given the early disappearance of this species the historic records are scarce; but they indicate that cranes occurred all year around in the UK (Northcote 1980; Boisseau & Yalden 1998; Stanbury 2011). Indeed, there are almost 300 places named after the species; mainly associated with moors, wetlands and pools (Boisseau & Yalden 1998). Crane bones are also frequent in many archaeological sites widely distributed across the country (Boisseau & Yalden 1998). Furthermore, there is evidence that in medieval times cranes were a delicacy; the aristocracy used to offer them in feasts and banquets (Gurney 1921). This fact, combined with egg collection during the nesting period could explain why the crane population became so severely depleted. Some measures to protect wildfowl were implemented in 1534; egg collection from March to June was punishable with imprisonment and a fine (Gurney 1921). However, those regulations did not prevent the disappearance of the population in the subsequent years. The last record of a young individual in the UK dates from 1542 in Hickling, Norfolk (Gurney 1921; Stanbury 2011).

After the extirpation of the breeding population, cranes became rare and only visited the UK as vagrants; from 1773 to 1950 only 73 sightings were recorded (Naylor 1996). But, the situation changed in subsequent years and the number of cranes visiting the UK increased (Stanbury 2011). From 1950 to 1975, around 5-10 individuals were recorded annually, with the exception of 1963, when more than 500 birds were recorded during the autumn migration due to

unusual weather conditions (Harber 1964; Stanbury 2011). From 1975 onwards there was another substantial increase in cranes visiting the UK, with around 50 to 250 annual records (Stanbury 2011). These birds were probably following the Western Flyway than runs from North Africa and the Iberian Peninsula, where birds winter, to Scandinavia, where they breed (Fig 1.2, BirdLife-International 2015). The increase in cranes visiting the UK across the years may reflect the increase in the number of cranes in some parts of Europe (Stanbury 2011). For example, the number of cranes wintering in Extremadura in the Iberian Peninsula grew at 3% per annum in the early 1990s (Guzmán *et al.* 1998).

In 1979, more than 400 years after the extirpation of the breeding population, two cranes arrived and established in Horsey, a wetland area in the Norfolk Broads (52° 44' N, 1° 37' E). They were believed to be 2- or 3-year old subadults. These two birds attempted to breed in the area in 1981 and they managed to hatch one chick that was predated a month later. The following year the pair nested again, this time with a successful outcome, they fledged a single chick. In the next seven years they managed to fledge another two chicks, until they disappeared in the early 1990s during a harsh winter (Stanbury 2011). By then, there was another pair breeding in the area, believed to be the chicks fledged by the original pair in 1982 and 1983 (Stanbury 2011). In the next few years the population increased slowly: in 1996 it had increased to three pairs in the area, but the productivity was extremely low, with no chicks fledging between 1990 and 1996.

In 2001, a study about the viability of the breeding flock in Norfolk was published and the authors established that the mean time to population extinction was 16.7 years (Mathews & Macdonald 2001). To boost the chances

of population survival they suggested artificially increasing the size of the founder flock. In particular they considered reinforcing the population with a high number of individuals infrequently to be more efficient than introducing a few individuals more often, as the first approach would provide many mates simultaneously, increasing the number of potential pairs formed. However, for a fixed total input of birds, frequent supplementations with fewer cranes was more likely to increase the chances of the population surviving. Overall, they concluded that the best strategy was to maximize the number of birds released to reinforce the population, adding them frequently over a long period of time. They also suggested other complementary measures, such as reducing mortality; specifically reducing juvenile mortality that could have a large impact on the population trajectory, since adult mortality was already high. The models that the authors developed also showed that immigration could be playing a role in the flock persistence; models incorporating immigration resulted in improved population persistence than those assuming that the population was isolated.

Nevertheless, despite lack of intervention the population survived and grew in the following years. In the early 2000s there were five pairs in the Norfolk Broads. The population growth rate increased after 2005, with 13 pairs in 2010 and 25 in 2015. The productivity also increased to an average of 0.5 chicks per nesting pair after 1997. From 1981 to 2001 cranes nested only in the Norfolk Broads. However in 2002 one breeding territory became occupied in South Yorkshire and several breeding attempts occurred there in the subsequent years. Then, in 2007 another pair nested at Lakenheath in the Cambridgeshire Fens, a wetland area 60 km away from the Norfolk Broads, and the territory remained occupied in the following years. Surprisingly, in 2012, one pair nested

in Scotland, more than 400km away from the founder population. Nowadays, most of those areas remain occupied.

1.4 Reintroduction

In 2006, the Wildfowl & Wetlands Trust (WWT), the Royal Society for the Protection of Birds (RSPB) and Pensthorpe Conservation Trust formed a partnership, The Great Crane Project (GCP), to establish a second population of cranes in the UK. The main goal of the reintroduction was to guarantee the survival of the breeding population of cranes in the UK. The target was to establish a self-sustained resident population by 2015, and to establish a new population of 20 pairs by 2030. In addition, there were secondary benefits: (1) cranes are flagship species and their population recovery would boost the conservation of wetlands and encourage conservation-friendly land use; (2) it would also engage land managers in habitat restoration; (3) the project approach would strengthen the cooperation between conservation organizations and (4) it would also generate popular support and publicity for conservation (Newbery *et al.* 2008).

After an assessment of potential reintroduction sites within the UK, cranes were translocated into the Somerset Levels (51° 2' N, 2° 55' W; 56,650 ha), a floodplain formed by wetlands, natural or semi-improved grasslands and arable fields of cereals and maize. The Somerset Levels were chosen because: (1) they are rich in invertebrate fauna, particularly those associated with the aquatic environment; (2) they have an extensive ditch system, open water, swamp and reed fringe habitats suitable for nesting; (3) there is active control and maintenance of water levels during nesting season; (4) there are large

protected areas managed by nature conservation organisations; (5) there is a high proportion of land owners following agri-environment schemes and (6) the area is ca. 250 km away from the existing crane populations and it has surrounding areas where cranes could potentially colonize. Nevertheless the site presented some minor drawbacks: (1) there is a Royal Navy's airfield and helicopter training over the area; (2) apparently there are high numbers of predators, particularly fox and (3) winter food resources might be scarce (Newbery *et al.* 2008).

Crane reintroduction in the Somerset Levels occurred from 2010 to 2014. For five consecutive years, up to 30 eggs were harvested in spring from a wild crane population in the Schorfheide-Chorin Biosphere Reserve (53° 1' N, 13° 47' E, Germany). They were transported to WWT Slimbridge Wetland Centre (51° 44' N, 2° 24' W) in the UK, where they were incubated and hatched. Chicks were hand-reared following techniques developed by the International Crane Federation in the USA to rear Sandhill and Whooping cranes (Newbery *et al.* 2008). Approximately ten weeks after hatching, chicks were transported to a release enclosure in Somerset where they spent a month prior to the release. Over the five year period, 94 birds were released in the site, 21 in 2010, 17 in 2011, 19 in 2012, 20 in 2013 and 17 in 2014. After the release, birds were monitored following the IUCN guidelines, which included: (1) colour ringing of all released birds; (2) tagging all individuals with radio, satellite or GPS transmitters and (3) monitoring birds' survival, breeding and feeding and roosting activity.

1.5 Thesis outline

The overarching goal of this thesis is twofold: to understand and predict the population dynamics and distribution of cranes in the UK and to assess people perception of conservation, using the crane reintroduction as an example. Specifically, in **chapter 2** I explore the potential of two Internet-based methods, offsite and onsite metrics, to understand the drivers of attitudes towards conservation. To do so, I use four examples: the pattern of interest in (i) the red kite *Milvus milvus* and (ii) the Royal Society for the Protection of Birds (RSPB), (iii) the public response to the first successful breeding of cranes in SW England in over 400 years and (iv) the use of the Internet by people to obtain information about conservation actions carried out by the Wildfowl & Wetlands Trust (WWT). In **chapter 3** I focus on the demography of the crane population. I try to disentangle the differential effects of survival, productivity and dispersal on population growth and understand the effect of the reintroduction on the population dynamics. In addition, I calculate the probability that the crane population will improve its conservation status in the next 50 years. On the other hand, in **chapter 4** I use a recently developed technique, spatio-temporal log-Gaussian Cox process models in a new framework, Integrated Nested Laplace Approximation methods (INLA), to determine wetland characteristics affecting crane recolonization, to predict the most likely wetlands to be colonized in the near future and to assess the effect of the reintroduction on the recolonization trends. Moreover, I explore the potential of those techniques to determine the spatial distribution for expanding populations where information about life-history traits, such as dispersion, is lacking. In **chapter 5** I examine the coping mechanisms of cranes in the face of an extreme flooding event and the consequences at a population level. Finally, in **chapter 6** I integrate the most

relevant results of the thesis and their wider implications for the conservation of small populations.

Chapter 2

Internet-based monitoring of public perception of conservation

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2.1 Abstract

Monitoring public perception of conservation is essential to ensure successful conservation outcomes. However, evaluating attitudes towards conservation projects presents daunting challenges because it is time consuming, expensive and open to social biases and small sample-size errors. Here, we present a recently developed approach to overcome these limitations – Internet-based methods - in particular offsite and onsite metrics. Offsite methods refer to Internet data mining tools that extract Internet search queries, such as Google Trends, while onsite methods refer to programmes that monitor traffic within websites, such as Google Analytics. We explore the potential of these methods rather than focus on the particular details of the case-studies provided to illustrate them. We used offsite methods to determine patterns in public interest in a reintroduced flagship species and in conservation awareness projects in the UK. We employed onsite metrics to assess the success in communicating a conservation outcome and to evaluate the success in online public engagement of a conservation NGO. Our results indicate that both offsite and onsite metrics are able to track changes in public interest across time and space. In particular, onsite metrics provide high levels of temporal and spatial resolution with a high degree of flexibility. These tools could add reliable information to traditional social surveys and represent an opportunity to improve our understanding of the drivers of interest in conservation.

2.2 Introduction

Public engagement is a fundamental part of effective conservation (Bowen-Jones & Entwistle 2002; Fischer *et al.* 2011). Firstly, public attitudes towards

environmental programmes can be major policy drivers and can ultimately influence the outcomes for biodiversity (Martín-López *et al.* 2009). Secondly, the engagement of local communities in conservation projects is often a key factor leading to successful implementation (Bowen-Jones & Entwistle 2002). However, evaluating public responses to environmental projects is challenging as most information is based upon public surveys, which are costly, time consuming and often suffer from small sample-sizes (Infield 1988; Newmark *et al.* 1993; Schlegel & Rupf 2010). Additionally, questionnaire responses can be difficult to interpret as a consequence of social context; for example because of non-response biases or social-desirability biases (Fisher 1993; Groves 2006).

The global extent of Internet use means that an increasing number of data sources are available to scientists to explore stakeholder opinion in ways that remove many of the biases associated with conventional approaches. Internet policies protect users' identity to an extent, and this feeling of anonymity may increase honesty (Solomon *et al.* 2007; Blank & Gavin 2009; Razafimanahaka *et al.* 2012). Moreover, the ubiquity of the Internet provides a wide range of Internet user profiles that encompass, to some degree, all possible demographic and social-economic groups, and allow coverage of extremely large geographic ranges. It is important to note, however, that Internet use might be lower in developing countries and among elderly people. Internet also provides wide temporal ranges; in Google Trends up to 12 years of data can be obtained, which is useful for assessing contemporary trends in ecological thinking (McCallum & Bury 2013; Lineman *et al.* 2015; Nghiem *et al.* 2016). Additionally, it produces massive sample sizes that are not available with traditional social surveys. The two main methods to exploit this data are offsite and onsite metrics. The first, offsite metrics, refers to programmes designed to

data mine the Internet and obtain information automatically about particular queries submitted to search engines, such as Google Trends, Naver Data Lab, Bing Trends or Baidu Trends; each one retrieves information from the respective search engine. The second, onsite metrics, refers to tools that monitor traffic within websites, such as Google Analytics, Twitter Analytics or Wikipedia Analytics. While Google Analytics can be implemented in any website, Twitter and Wikipedia Analytics are embedded in their respective websites.

In some fields of study, offsite metrics measuring Internet search behaviour have been widely applied to assess population interest. For example, in economics this technique has been used to forecast consumer habits or to obtain indicators of the level of economic activity with shorter time lags than traditional methods (Vosen & Schmidt, 2011; Choi & Varian 2012). Moreover, information from search engines has been used in medicine to monitor health issues such as disease outbreaks and suicide risk (Carneiro & Mylonakis 2009; Pelat *et al.* 2009; McCarthy 2010). Despite the clear potential of offsite metrics (reviewed in Ladle *et al.* 2016), it has only been applied in a small number of conservation studies to estimate temporal trends in interest in general environmental concerns (McCallum & Bury 2013; Lineman *et al.* 2015; Nghiem *et al.* 2016), to assess change in interest in wetlands after their protection (Do *et al.* 2015), to determine trends for general fishing related terms (Martin *et al.* 2012; Wilde & Pope 2013), to track biological processes and invasive species (Proulx *et al.* 2014; Szymkowiak & Kuczynski 2015) or to assess species popularity in relation to their characteristics (Correia *et al.* 2016).

Onsite metrics, while being used to evaluate the usability of e-commerce sites or the potential of online health interventions (Hasan *et al.* 2009; Crutzen *et al.* 2012), have been overlooked as tools to assess public engagement in conservation. Such onsite web analytical services provide fine-grained information about online users' behaviour within a website, such as where they arrived from, which pages they visited and for how long, and the route taken through a website. To extract conclusions about differential preferences this information can be combined with social and demographic information. For example, comparison of traffic intensity among projects would indicate which are more engaging. In addition, onsite metrics could be used to validate whether projects targeting certain sections of the population are succeeding. More interestingly, if this is followed by actions designed to increase interest in certain projects, web traffic becomes an advantageous tool to determine the efficacy of those actions.

Onsite and offsite metrics can act as complementary tools since they measure different aspects of Internet use. In general onsite metrics produce a wider range of information and more detailed outputs (see onsite metrics section for detailed explanation). Moreover, since they are embedded in the website they can glean information at much lower traffic volumes than would be possible using offsite metrics, which only provide the data if the search volume exceeds a threshold (Table 2.1). The main drawback of onsite metrics is that they require a certain programming knowledge to be implemented and a website has to be developed (Table 2.1).

Table 2.1: Comparison between offsite and onsite metrics characteristics

	OFFSITE METRICS	ONSITE METRICS
Data accessibility	Data is public and available through several data mining tools, such as Google Trends	Data is private and the programme (e.g. Google Analytics) needs to be implemented in the website
Data type	Relative search volumes, i.e. search volume relative to the highest point in the term's popularity	Absolute number of visits to the website
Data availability	Only searches that reach a certain volume threshold can be retrieved	All data can be retrieved
Repeatability	Data is generated through non-public algorithms that might be modified, thus the same search can yield different results if the algorithms change	The results from onsite metrics are not generated by any algorithm, thus the data is more reliable
Time span	Depends on the data mining tool; Google Trends data are available from 2004 onwards	Data can be retrieved only after implementing the programme in the website
Temporal resolution	Weekly	Hourly
Geographic resolution	From country to town, aggregated in specific period for search terms with high volume	From country to town, easily aggregated in specified periods
Representability	Capture all the traffic going through a particular search engine	Capture all the traffic going through a particular website
Demographic information	None	Estimate ages and gender of visitors through third party cookies

Here we aim to introduce and illustrate the opportunities provided by Internet-based datasets to assess public interest in conservation. Specifically, we examine the potential for conservation NGOs, managers and academics to quantify the interest in particular projects and the aspect of those projects that engage the public most. Our goal is to explain the potential of these tools and provide a framework for their use rather than focus on the detail of the case-

studies chosen to illustrate them. First, we highlight the opportunities offered by offsite metrics through two examples. We assess patterns in public interest in a bird of prey, the red kite *Milvus milvus*, in the United Kingdom (UK); we selected this species because, after near extirpation, several geographically discrete reintroductions have been conducted, during which public engagement was a major priority. We then investigate temporal patterns of public interest in the *Royal Society for the Protection of Birds* (RSPB, <http://www.rspb.org.uk/>), one of the biggest conservation NGOs in the UK. Second, we demonstrate the use of onsite metrics by analysing the effect of a single conservation outcome from a different reintroduction project, *The Great Crane Project* (GCP, <http://www.thegreatcraneproject.org.uk/>), that successfully returned Eurasian cranes *Grus grus* to SW England 400 years after their local extirpation. We also analyse the importance of the Internet in communicating conservation actions using data from the *Wildfowl & Wetlands Trust* (WWT, <http://www.wwt.org.uk/>), an NGO focussed on the conservation of wetlands.

2.3 Offsite metrics

We chose a Google tool for our study as it is the most used search engine, with over 40,000 search queries per second (<http://www.Internetlivestats.com/>). Google Trends reports relative rather than absolute search volumes on a 0–100 scale, i.e. for any given time-period, it yields the search volume relative to the highest point in popularity (= 100) of that term over the whole time-period under consideration. If we add a second term to the search, its popularity as a search term is measured on the same scale as the first term, thus allowing comparison of the popularity of the two terms. A decreasing trend in the index does not

necessarily imply a decrease in the absolute number of searches (although this could be the case), but it does mean a decrease in the search term's popularity compared to other searches. This is one of the main weaknesses of the tool and has been raised as an issue in studies that assess whether interest in the natural environment is decreasing (Ficetola 2013; McCallum & Bury 2013; McCallum & Bury 2014). Nevertheless, there is ample evidence that Google Trends outputs are generally good indicators of public interest in areas like epidemiology and general public opinion (Ginsberg *et al.* 2009; Ripberger 2011; Scharkow & Vogelgesang 2011; McCallum & Bury 2014). An additional limitation is that, in cases where the volume of searches only breaks the threshold during certain time periods, any trend can be difficult to interpret. This means that Google Trends is best suited to assess high impact conservation projects (e.g. red kite reintroduction) or the activities of larger conservation organisations such as the RSPB.

2.3.1 *Assessing patterns of public interest in a species: the red kite*

Being able to target the periods and areas where people are more interested in certain species and conservation projects is crucial in the development of successful awareness campaigns. In this case, we present the seasonal pattern of interest in the red kite, a species which has been reintroduced in the UK (Fig. 2.1A). We selected this species because it is regarded as a flagship in the UK and many conservation actions have taken place to aid population recovery. Red kites were reintroduced in the UK in 10 areas across the country between 1989 and 2010 (Fig. 2.1B). We queried the search term red kite and observed a distinct and seasonal pattern: in May and June (i.e. during the main breeding period) we obtained the highest indexes of popularity within the year and this

pattern was repeated across years (Fig. 2.1A). To support the idea that the seasonal variation in interest in the red kite could be driven by similar underlying processes to the interest in breeding, we queried the term nesting. We calculated the Spearman's correlation coefficient between both terms and observed that both terms presented almost identical trends ($r_s = 0.77$, $P < 0.001$, Fig. 2.1A). This increase in interest during the breeding period could be due to the birds being more visible to the public at this time; the birds perform impressive flight displays in May when occupying their breeding territories. In addition, seasonal improvement in weather could lead to an increase in outdoor (e.g. birdwatching) activities. Alternatively, regardless of whether people actually see the birds, there could be an interest in the breeding success of the population, or a peak in media releases related to the success of the breeding season.

We also mapped geographic interest in the red kite and found that relative search volumes for the term were higher in the areas where the reintroductions took place (Fig. 2.1B). For instance, the highest relative search volume was in High Wycombe, where red kites were reintroduced during the 1990s (Carter & Grice 2000). We also observed that for some areas of Scotland where reintroductions took place no search volumes were detected, likely linked to the low human population densities in those regions and highlighting the threshold issue outlined above.

These results demonstrate that offsite metrics can detect both spatial and temporal patterns of interest in a conservation project. This type of approach could help pinpoint the types of activity that generate most interest among the public (temporal patterns) and also identify regions/localities where despite

conservation actions being initiated public interest is lower than expected. However, to truly understand these latter patterns, human population density needs to be taken into account, as in rural areas with low population it is likely that relative search volumes will not reach the required threshold for the data to be provided.

This approach also allows conservation managers to query topics in Google Trends before and after the conservation action takes place in order to assess whether interest has changed and how any change varies spatially. At the moment, to assess the variation at a local scale over time for terms with relative low search volumes, such as red kite, it is necessary to retrieve the information separately for different segments of the period of interest, since Google Trends only provides the accumulated interest over the whole period.

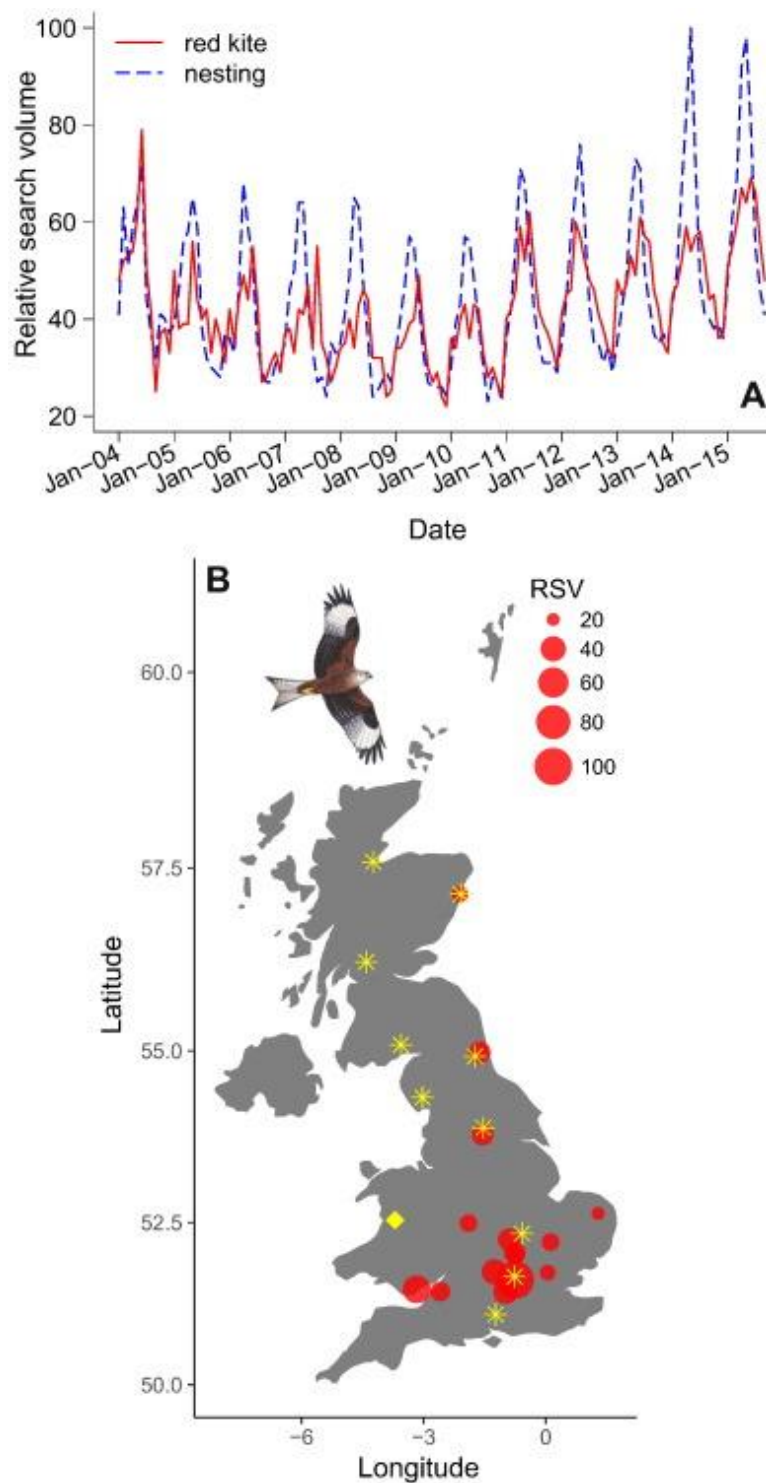


Fig 2.1: Google Trends relative search volumes for (A) *red kite* and *nesting* from January 2004 to September 2015 in the UK and (B) *red kite* geographic relative search volumes for the same period of time. The diamond indicates location of the natural population of red kites. Asterisks indicate locations of the reintroduced populations.

2.3.2 *Assessing patterns of interest in conservation awareness projects: RSPB*

To assess patterns of interest in conservation initiatives, we investigated the types of project that influence public interest in conservation NGOs. We used Google Trends to query: *RSPB* (the conservation NGO), *Big Garden Birdwatch* and *bird identification* for the UK. The Big Garden Birdwatch is an RSPB citizen science project that takes place every January and encourages people to count birds in their gardens. This project has been running for over 30 years and in 2015 over half a million people participated (<http://www.rspb.org.uk/>). The *bird identification* query leads the search to a tool developed by the RSPB, a bird guide to identify common British birds. Our results indicate that timings of searches for *RSPB* are significantly correlated with searches for *Big Garden Birdwatch* (Spearman's Rank Correlation, $r_s = 0.48$, $P < 0.001$, Fig. 2.2) and *bird identification* ($r_s = 0.19$, $P = 0.021$, Fig. 2.2), which indicates that interest in the RSPB website is driven by both projects. Our results suggest that the Big Garden Birdwatch programme might be increasing the popularity of the RSPB website; an impressive impact for a single project. Similar to the red kites, interest in the *bird identification* tool has a seasonal pattern as well; with higher values in spring, when birds start breeding and the weather improves and so outdoor activities, such as birdwatching, increase. We found that Google Trends is a powerful tool to measure the success of projects, which is essential to develop optimal strategies to increase both awareness and donations.

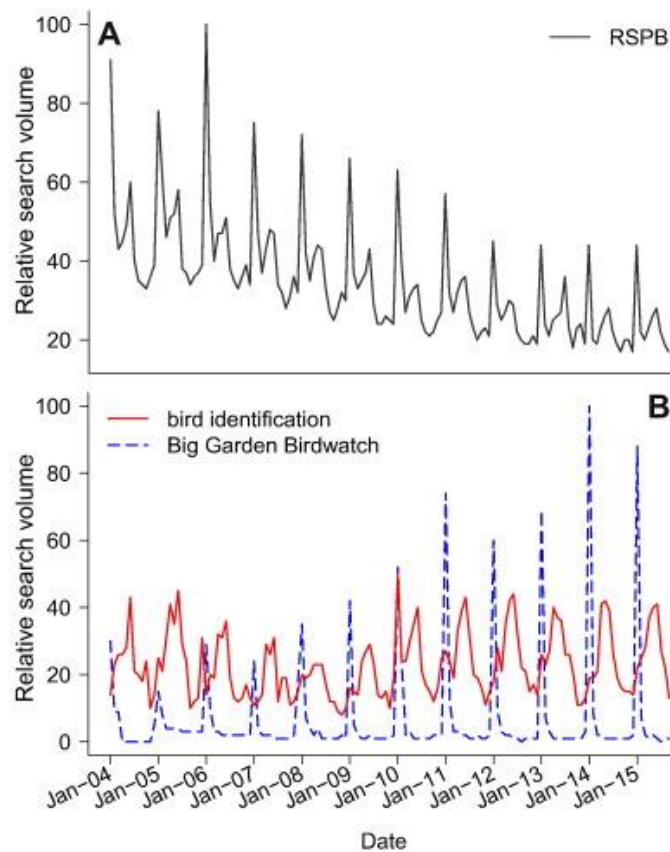


Fig 2.2: Google Trends relative search volumes for (A) *RSPB* and (B) *bird identification* and *Big Garden Birdwatch* from January 2004 to September 2015 in the UK.

2.4 Onsite metrics

Onsite metrics refer to tools that monitor traffic within a website. We used Google Analytics, a web service provided by Google that only requires the insertion of some code into each webpage to allow web traffic to be followed. This service provides a range of information: the number of visitors to the website during a certain time period (volume of visitors), as well as their bounce rate (percentage of people leaving the webpage without interacting with it), the number of pages visited within the website, time spent on each page, the channels used to access the website (social media, direct search, advertisement, etc.), landing page (the page of the website they first hit), or percentage of people accomplishing certain goals (such as joining the

organisation or making a donation). It can also provide linguistic and geographic distribution, from country to town level, as well as demographic proxies from the visitors such as gender or age. This information comes from third-party cookies and mobile apps; for example, in some cases demographic parameters are assessed based on the sites that have previously been visited or in other cases, they are provided by certain websites, such as social networking sites. Additionally, the tool allows the retrieval of information about user behaviour, such as the paths followed when navigating the website.

2.4.1 *Assessing the success in communicating conservation outcomes: the Eurasian crane*

To illustrate the power of onsite metrics relative to offsite metrics we analysed the impact of a media release in August 2015 that detailed the first successful breeding of Eurasian cranes in SW England in over 400 years. We monitored the traffic within the Great Crane Project (GCP) website (<http://www.thegreatcraneproject.org.uk/>) from the 1st of April to the 21st of December 2015 to assess the impact of the media release on public interest. In order to contrast these data with those gathered from offsite metrics we queried the topic crane (as a bird) in Google Trends during the same period. The day of the media release, the traffic on the GCP website increased six-fold, from 200 to 300 page views per day to over 1400 and was higher than usual, with 400–600 page views per day, for four days. During the subsequent days the web traffic returned to baseline values (Fig. 2.3A). Popularity in Google Trends also peaked during that period, but it was delayed by two weeks and the resolution was much poorer than the Google Analytics data set (Fig. 2.3B). This comparison highlights the degree of precision of both metrics: while Google

Analytics showed a clear and instantaneous response, the response in Google Trends was slightly delayed and the temporal pattern of search was not as clear. It is important also to note that although in this case the media release had detectable impact, lower impact media releases might not be apparent in Google Trends.

Google Analytics also allowed us to retrieve information about the geographic distribution of the people interested in the event. Such fine-scale spatio-temporal data is not generated by Google Trends, where geographic information at a city level was available only as a summary for the whole period 2004 to the present and could not be split into particular time periods due to the low volume of traffic for the term crane. We retrieved visitors' geographic distribution data for the day of the media release (number of visitors per locality) and weighted it by population size. We calculated the distance between visitors' geographic locations and the closest release site. Subsequently, we estimated the Spearman's correlation coefficient between the number of visitors per locality (weighted by population size) and the distance from the locality to the closest release site. In a similar fashion to the red kite data set, we found that towns close to the breeding sites had a relatively higher interest in the project ($r_s = -0.42$, $P < 0.001$, Fig. 2.3C).

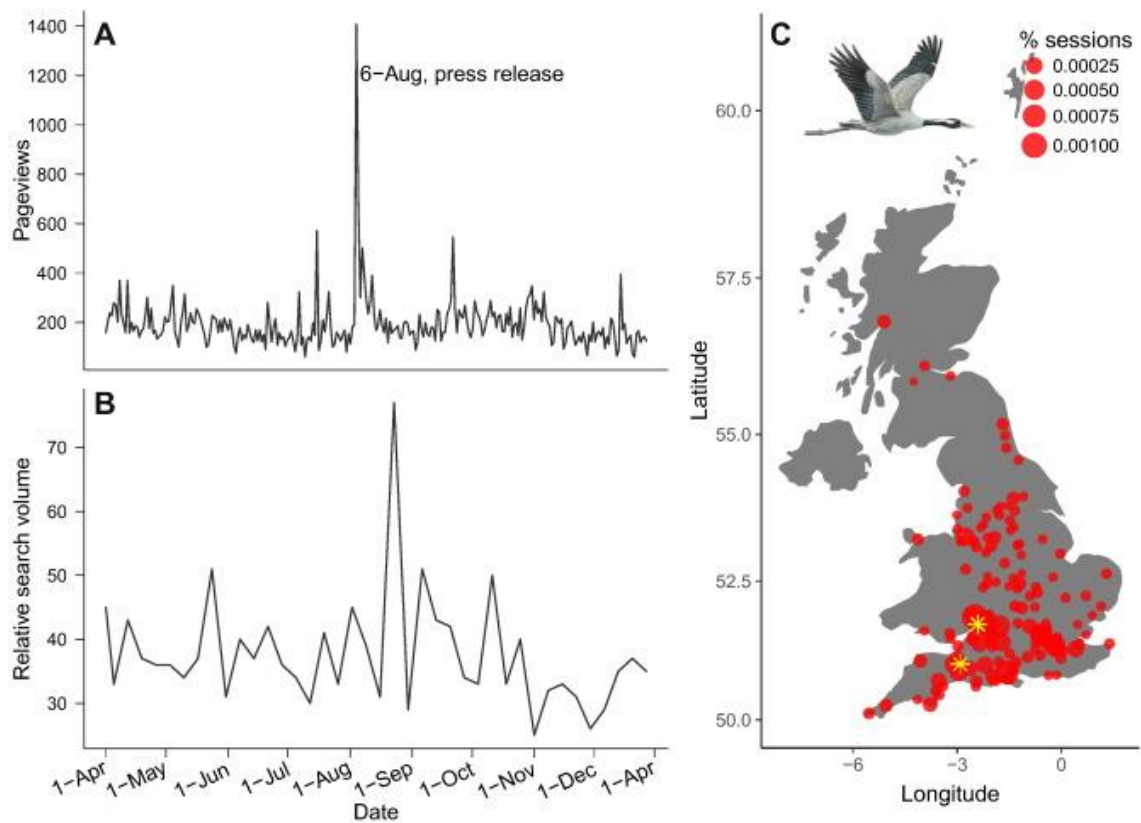


Fig 2.3: (A) Number of page views from the Great Crane Project website from April to December 2015; (B) Google Trends relative search volumes for crane (as a bird) from April to December 2015; and (C) geographic distribution of the Great Crane Project website visitors on the day of the media release. Asterisks indicate locations of the reintroduced population of Eurasian cranes.

This onsite approach clearly delivers detailed information on the magnitude of the impact of a conservation awareness action. In our case, we observe that there is an acute response to the media release but after several days the volume of visits to the website returns to previous values. Moreover, the number of returning visitors did not increase after the media release; it remained stable during the whole period (50-80% of returning visitors per day). This pattern perhaps indicates that transient public attraction to the project did not translate into sustained engagement by large numbers of people.

In this case, we found Google Analytics to be a very powerful tool for capturing subtle changes in interest that otherwise could be overlooked by more general tools such as Google Trends. An extension to our work would be to estimate the minimum frequency of media releases needed to increase the baseline number of visitors, since generating new online resources is time consuming and this information would allow better allocation of resources. Additionally, in the case that particular geographic communities were targeted in a conservation awareness project, the geographic distribution of the visitors would provide a proxy for project success. The same principle would apply if the target were specific demographic sectors since Google Analytics provides age and sex specific information, for example many conservation organisations are interested in engaging younger age groups.

2.4.2 *Assessing the success in online public engagement: WWT*

Understanding which channels are used by people to gain information on conservation is crucial to awareness and fund-raising for conservation NGOs. In this case study, our aim was to determine whether people use the Internet to obtain information about conservation actions carried out by the Wildfowl & Wetlands Trust (WWT). To do this, we used the WWT website, which is excellent for this kind of question because it is divided into two distinctive sections: one covers conservation projects (such as the reintroduction of the Eurasian Cranes) and the other focuses on the WWT wildlife reserves and visitor centres (Wetlands Centres). Since the access to information on the two areas is parallel within the website (i.e. both sections could be accessed from the main website page), we assumed that search behaviour would be due to interest rather than an artefact of website design. We analysed the web

behaviour from a random subsample of 5000 sessions (5.03% of the total) for the year 1st September 2014 to 31st August 2015. The number of sessions was imposed by Google Analytics since it is unable to process a higher number. Our results showed that after arriving on the home page, visitors clicked on the Wetland Centres information in 48% of the sessions, contrasting with only 5% of clicks for the conservation section (the remaining 47% chose other sections). However, 6% of the visitors that were first interested in the Wetland Centres also visited the conservation section afterwards. The average time spent on both sites was similar, 38 s on the conservation section and 40 on the Wetland Centres section. However, the bounce rate (percentage of people leaving the webpage without interacting with it) was higher (52.5%) in the conservation section, than the rate from the Wetland Centres section (29.7%), indicating that people interested in the wetland centres were more likely to do further research on the website and that a proportion of visitors to the conservation sections landed there by mistake, exiting the website promptly. These metrics provide a valuable insight into the nature of public interest in WWT and a baseline against which to measure future change. For example, if actions were taken to increase the visibility of WWT conservation projects, Google Analytics could determine whether use of particular elements of the web-site, such as the conservation sections (or even individual projects within these elements), increases over time. It would also be possible to gauge the outcomes of such behaviour, for example, whether visitors to conservation project web pages contribute an increasing proportion of new memberships or donations to the organisation.

In order to answer more complex questions about conservation through visitor online behaviour we make a call for a closer collaboration among conservationists, behavioural psychologists and web developers. An efficient

design of a website would not only provide useful information to the visitors through its online resources but also to conservationists through onsite metrics.

2.5 Conclusions

Our study illustrates that Internet-based tools for monitoring user activity can provide a flexible and detailed approach to assessing public interest in a wide range of conservation topics and projects. To date, Google Trends has only been used to study broader aspects of public awareness of conservation (McCallum & Bury 2013; Proulx *et al.* 2014). However, our results indicate that both Google Trends and Analytics can be also used to answer specific questions, such as the degree of success in engaging people in various aspects of conservation, with good spatial and temporal resolution. The potential of these tools is enormous, and they could be used to assess a wide range of topics, such as, which flagship species are most popular, how people respond to media releases and information dissemination and the geographic range reached by publicity activities. Nevertheless, the use of onsite or offsite metrics will depend on the question asked, since both methods have different specifications (Table 2.1); while offsite metrics cover all the traffic of a particular search engine, onsite metrics only cover the traffic through a particular website. The advantage of onsite metrics is that they offer a higher level of detail and more reliable results since they do not rely on opaque algorithms that might change over time, yielding different relative search volumes for the same term during the same time period if the data is retrieved at different points in time. In fact, when we retrieved the Google Trends relative search volumes for crane, from April to December 2015, two years later (2017), the pattern had changed

and the peak after the press release was no longer apparent. Thus, we suggest that onsite metrics open new possibilities, ranging from simple applications that are analogous to offsite metrics, to much more complex approaches, where web design could be combined with specific human behaviour experiments to grasp subtle drivers of the behaviour of people using these web sites and how this reflects or informs their interest in conservation. We encourage conservation managers to consider using Internet-based tools to monitor whether their projects are having the desired effect on the public perception of conservation.

Chapter 3

Immigration and reinforcement drive population dynamics in a long-lived bird: Implications for Eurasian crane conservation

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3.1 Abstract

1. Understanding population dynamics and designing appropriate management strategies requires knowledge of the differential effects of survival, productivity and dispersal in population growth.
2. This is particularly the case for small and recently established populations, where stochastic birth and death rates may end up producing sinks and population persistence relying on net immigration. Reinforcement of populations with additional individuals is therefore becoming a common strategy to boost the size of these small populations.
3. Here, we investigated the importance of population reinforcement in a recently established population of Eurasian cranes (*Grus grus*) in the UK. We developed stochastic population models to assess the population dynamics and the effect of adding 90 individuals between 2010 and 2014, while also quantifying the population-level effects of immigration.
4. The best-supported models suggest that the crane population is self-sustaining with an annual adult survival of 0.88; however much of the population increase observed in recent years has been driven by immigration of birds from continental Europe.
5. We found that population reinforcement resulted in a 50% increase in the projected population size from 178 to 275 breeding pairs over the next 50 years. We also found that the relative contribution of immigration to population growth declined from 43%, when the reintroduced birds were not considered, to 29%, when they were included in the breeding pool. Moreover, after the population reinforcement, the probability of the

population improving its conservation status increased from just above zero to 32%.

6. *Synthesis and applications.* Population reinforcement is a highly effective technique to boost numbers in small and slowly growing populations. In our case, a modest reintroduction programme resulted in a 50% increase in the projected population size and a reduction in the long-term dependency on immigration. The reintroduction has the added value that an increased breeding pool would decrease population instability associated with demographic stochasticity in small populations.

3.2 Introduction

An understanding of the processes that influence demography lies at the heart of successful conservation and is a prerequisite to the implementation of management strategies that enhance the survival prospects of a population (Levin *et al.* 2009). Disentangling the effects of survival, productivity and dispersal on population dynamics is imperative, since these factors may contribute differentially to population growth and can mask important, but cryptic, demographic processes, such as source-sink dynamics (Schaub *et al.* 2010; Schaub, Jakober & Stauber 2013; Lieury *et al.* 2016; Sanz-Aguilar *et al.* 2016; Weegman *et al.* 2016). Until recently it was commonly assumed that sink populations could only last for relatively short periods of time, but recent evidence suggests that, in some cases, source-sink dynamics can be sustained for prolonged periods, jeopardizing the assumption that persistent populations are always acting as sources (Schaub *et al.* 2010; Schaub, Jakober & Stauber 2013; Lieury *et al.* 2016; Sanz-Aguilar *et al.* 2016; Weegman *et al.* 2016). This means that persistence or stability of numbers are not good indicators of

population health and discriminating among sources (populations in which births exceed deaths and have net emigration), sinks (populations in which deaths exceed births but that persist through net immigration) and pseudosinks (populations that have both net immigration and net recruitment; Pulliam 1988; Watkinson & Sutherland 1995) should form an essential component of any population recovery programme.

Among small, recently established, populations the role of immigration in population growth is likely to be particularly important as births and deaths are often highly stochastic (Lande, Engen & Saether 2003). In such cases, if immigration ceases, populations can stop growing or even move towards extinction (Andreassen & Ims 2001; Schaub, Jakober & Stauber 2013; Altwegg, Jenkins & Abadi 2014; Tauler *et al.* 2015). Thus, an efficient management strategy for small populations is the reinforcement of the population with additional individuals, since it would increase the breeding pool and in turn the total annual productivity of the population (Armstrong & Seddon 2008). Population reinforcements have been previously proposed to increase size in small reintroduced populations (Vandel *et al.* 2006), to avoid inbreeding depression and maintain gene flow (Gusset *et al.* 2009; King, Chamberlan & Courage 2014; Bull *et al.* 2016), or to overcome barriers to dispersal from other populations (Seddon, Strauss & Innes 2012). In addition, in some cases, reinforcement programmes have been implemented in populations that are not self-sustaining to delay extirpation while additional conservation measures to boost productivity and reduced mortality are enforced (Keedwell, Maloney & Murray 2002). To our knowledge, however, no study has investigated the potential effect of population reinforcement on source-sink dynamics and their consequence in terms of population recovery.

In this study, we investigate these two processes, source-sink dynamics and population reinforcement, in a population of Eurasian cranes (*Grus grus*, hereafter cranes) in the UK. Historic records indicate that cranes were common resident birds in the UK until the 16th century, when hunting and egg harvesting drove the population to national extirpation (Northcote 1980; Boisseau & Yalden 1998; Stanbury 2011). However, in 1979 a breeding pair established itself on a large wetland in south-eastern UK (52° 36' N, 1° 36' E). Following this colonisation event, the population persisted but barely increased: by 1996 it had risen to only three pairs (Stanbury 2011). One of the reasons for the lack of increase appears to be that productivity was extremely low and no chicks fledged between 1990 and 1996 (Mathews & Macdonald 2001; Stanbury 2011). In 2001, a population viability analysis (PVA) predicted the mean time to extinction for this newly established population was around 17 years (Mathews & Macdonald 2001). Despite this, the population increased from the late 1990s onwards, reaching 20 breeding pairs in 2015, which have colonized several wetland areas across the eastern UK (Stanbury 2011; Soriano-Redondo *et al.* unpublished).

In order to enhance the likelihood of the UK breeding population persisting, a reintroduction programme was initiated. Between 2010 and 2014, 90 juvenile birds (46 females and 44 males) from a German population were released into the Somerset Levels (51° 2' N, 2° 55' W), a wetland area situated in SW England, ca. 250 km away from the naturally established population (Soriano-Redondo *et al.* 2016). Although this reintroduction was initially seen as independent of the natural population, there is already connectivity between the two populations and a pair comprising one bird from each population bred in 2015 (Soriano-Redondo *et al.* unpublished). We expect the population mixing to

increase in the near future as reintroduced cranes reach maturity and establish new breeding territories away from their release site (Soriano-Redondo *et al.* unpublished). In addition, the natural population has slightly expanded its range westwards in the last 15 years (Stanbury 2011; Soriano-Redondo *et al.* unpublished). Thus, the crane reintroduction programme can now be viewed as a reinforcement of the natural population in terms of population dynamics.

In this study, our overarching goal is to quantify the population dynamics of cranes in the UK and evaluate the potential effect of the reinforcement on population growth. Specifically, because the UK was recolonized by birds from continental Europe (Mathews & Macdonald 2001; Stanbury 2011), we test whether the population is self-sustaining or a cryptic sink in which immigrants from continental Europe make the main contribution to population growth. In addition, we assess the probability that the population's conservation status will improve in the UK Red List for Birds (from *amber* to *green* (Eaton *et al.* 2015) in the future. To do this, we use stochastic matrix population models to provide important insights into the potential benefits of the population reinforcement for small but recovering populations.

3.3 Methods

3.3.1 Data collection

Data on crane breeding was gathered from a range of sources across the UK: landowners, site-managers, the Rare Breeding Birds Panel (<http://www.rbbp.org.uk/>), and the Scarce Migrant Reports in the journal *British Birds*. Cranes are large and very recognisable birds and it is unlikely any

breeding attempt was missed. We considered the following criteria as evidence of breeding: (1) an incubating bird or a chick was seen, (2) there was post-breeding evidence of eggs being laid or young being present, (3) a crane family was seen post-fledging or (4) adults were acting in a manner that strongly suggested the presence of a nest or young. Breeding success and number of offspring produced were assessed through direct observation once the chicks had fledged.

3.3.2 *Model parameters and stochastic matrix population models implementation*

Cranes are monogamous birds with delayed sexual maturity; the age at first breeding is estimated as four years for both sexes (Cramp & Simmons 1983). To implement stochastic matrix population models and maximum likelihood methods (details below) we defined the species life cycle and calculated the vital rates of the population. We used a life cycle with five age classes: fledgling, one year old sub-adult, two-year-old sub-adult, three-year-old sub-adult and adult (Cramp & Simmons 1983; Johnsgard 1983; Mathews & Macdonald 2001). We based the analysis on the post-reproductive census, restricted to females. We assumed equal sex ratio and recruitment at four years old (Cramp & Simmons 1983; Mathews & Macdonald 2001).

There have been a few attempts to develop demographic models for the Eurasian crane in Europe (Alonso *et al.* 1986; Alonso *et al.* 1991). These models indicate that crane populations are resilient and able to cope with low levels of hunting and reduced productivity. Nevertheless, those models present some limitations: they only cover a relatively short-time period (8 years), survival data are lacking and demographic and environmental stochasticity are

not modelled. Unfortunately, at present, the lack of survival data available on Eurasian cranes is still an issue, thus we used age-specific survival estimates for immature birds from sandhill cranes (*Grus canadensis*), a North American congener with similar ecology and body size (Hayes & Barzen 2016). Estimates of annual survival rate for fledglings were 0.78 and for birds in the three sub-adult age classes were 0.91 (Hayes & Barzen 2016). Given that data came from a surrogate species, and the high sub-adult survival of sandhill cranes, we ran additional models in which sub-adult survival rate was reduced by 2% to test the robustness of our results. The stable-stage distribution provided an initial population vector in 2000 of $n_0 = (1, 1, 1, 1, 3)$, each number corresponding to the number of females in each age class.

To calculate the population growth rate (λ) and productivity we focused on the period between 2000 and 2015 because of the distinct historic dynamics of the UK population; from 1981 to 2000 the population numbers were extremely low and rather stable which contrasts with the increase from 2000 onwards. Population growth rate was based on the observed number of breeding pairs for the 16-year period, and the productivity was calculated as the average of the annual number of fledglings produced divided by the number of breeding pairs. We considered that most territorial non-breeding pairs (i.e. pairs holding territories but showing none of the signs of breeding outlined above) were formed by sub-adults that were in the early stages of pairing and therefore should not be represented in the breeding adult female pool; however, it is possible that some of these pairs comprised young adults. Thus, we performed a second set of simulations including females from the non-breeding pairs in the breeding pool to assess the extent to which this potential source of error might influence our models.

We constructed stochastic matrix population models with Monte Carlo simulations to account for environmental and demographic stochasticity (White 2000; Morris & Doak 2002). We used the stretched beta and the beta distribution to simulate environmental variability for productivity and adult survival respectively (package POPBIO, ver. 2.4.3., Morris & Doak 2002; Stubben & Milligan 2007). To account for demographic stochasticity, we first calculated the proportion of pairs having zero, one or two fledglings for every year, and then we correlated those proportions with the annual productivity. Second, we obtained a productivity value from the stretched beta distribution and predicted the total number of fledglings raised each year using a multinomial distribution (zero, one or two fledglings per pair). To assess the most likely adult survival and immigration estimates we ran a set of 60 models with unique values of survival (12 survival scenarios) and immigration (5 immigration scenarios), with 10,000 replicates per model. We ran all possible combinations for adult survival ranging from 0.84 to 0.95 (0.01 intervals) and immigration ranging from zero to four females per year. We constrained the possible adult survival around 0.84-0.95 since most evidence supports high adult survival in cranes (Johnsgard 1983; Mathews & Macdonald 2001; Hayes & Barzen 2016); and we chose a maximum of four females per year since empirical data would suggest that this is already a large overestimate of the actual immigration (Stanbury 2011). We considered the best supported model to be the one obtaining the closest match to the observed lambda (see Hernández-Matías *et al.* 2013 and Tauler *et al.* 2015 for further details). All analyses were performed using R ver. 3.3.0 statistical software (R Development Core Team).

3.3.3 *Population viability analysis under different immigration and reintroduction scenarios*

We ran four forward-in-time demographic simulations combining different immigration and reintroduction scenarios to understand the relative contribution in the population growth of both immigration and population reinforcement. The first corresponded to the best-supported model from the analysis above without considering the reintroduced birds; the second corresponded to the best-supported model considering the reintroduced birds. Finally, we simulated two scenarios including and excluding the reintroduced birds with the best-supported adult survival but assuming no immigrants were supplementing the population. For the demographic simulations that excluded the reintroduced birds the initial population vector considered in 2015 was $n_0 = (10, 5, 5, 5, 20)$, based on the stable-stage distribution of the population, since we only had information on the number of breeding females. For the two simulations that accounted for the reintroduced birds we included into the initial population vector the 40 female released birds that had survived to 2015; eight female birds were one-year-old, ten were two years old, six were three years old and 16 were adults, yielding an initial population of $n_0 = (10, 13, 15, 11, 36)$. For each model, we simulated 10,000 replicates with a 50-year time-horizon. To calculate the probability of the population reaching 300 breeding pairs in 50 years, the criterion for cranes to be listed as non-threatened (*green* status) in the UK Red List for Birds (Eaton *et al.* 2015), we determined the proportion of the 10,000 replicates that reached 300 pairs.

3.4 Results

3.4.1 *Model parameters and stochastic matrix population models implementation*

Between 1981 and 2000 the population of breeding cranes in the UK barely changed, increasing only from one to three pairs. However, in the following 14 years (2000-2014) it reached 20 pairs, with a population growth rate of 1.13 and an annual productivity of 0.56 chicks per breeding pair. The set of 60 stochastic population models with sub-adult survival estimates obtained from sandhill cranes and adult survival ranging from 0.84 to 0.95 (0.01 intervals) and immigration from zero to four females per year yielded a best-supported model with 0.88 annual adult survival and one female immigrant per year (Figs 3.1 & 3.2); it predicted 19 adult females in 2015 vs. the observed 20 adult females (Fig 3.2).

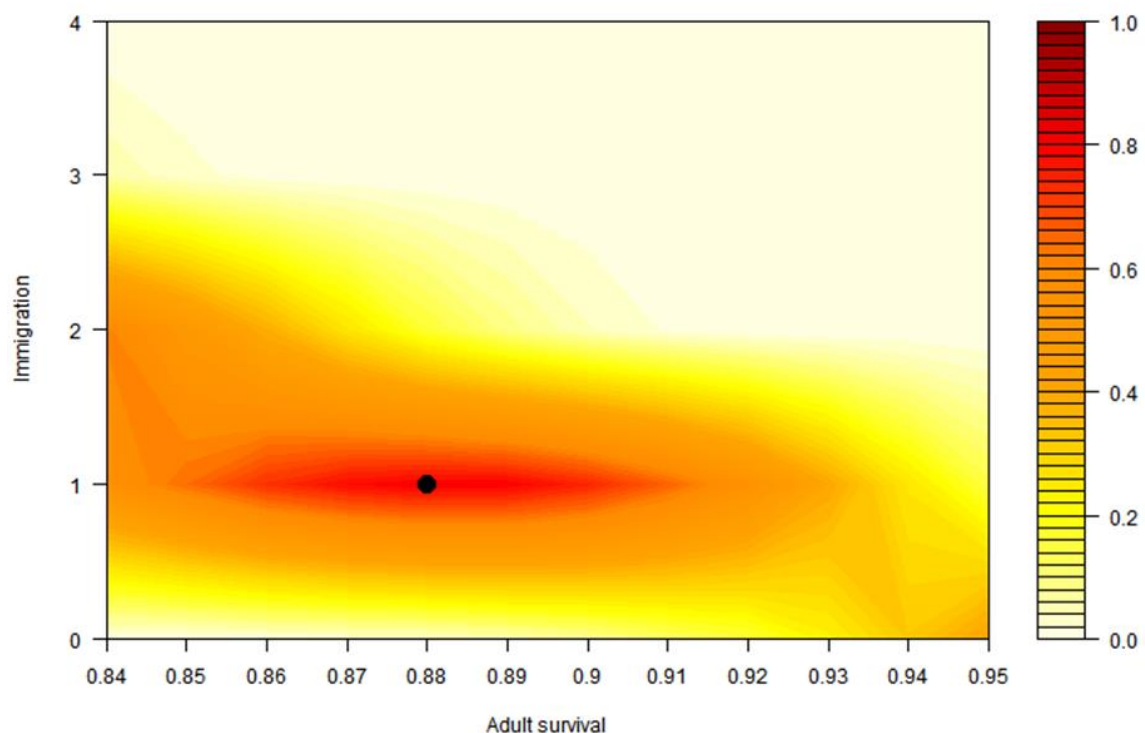


Fig 3.1: Likelihood estimate for a range of values of both adult survival and number of immigrants of cranes in the UK. Black dot indicates the combination with the highest likelihood, 0.88 adult survival and one female immigrant per year.

In the scenario with reduced sub-adult survival, the best-supported model yielded the exact same results, 0.88 annual adult survival and one female immigrant per year (Supplementary Fig. 3.S1). However, when we included the females from the non-breeding pairs into the breeding pool, the population growth rate showed a small decrease to 1.12 and productivity decreased more markedly to 0.46. For this suite of simulations, there were two highly supported models, the first with 0.87 annual adult survival and the second with 0.88. In both these cases immigration rates increased to two females per year (Supplementary Fig. 3.S2). Since best-fit adult survival was consistent across all of the best supported models and the model excluding the non-breeding females produced a better prediction of the observed population size (19 predicted adult females vs. 20 observed adult females, Fig 3.2), than the model including the territorial non-breeding females (31 predicted adult females vs. 25 observed adult females, Supplementary Fig. 3.S3), we decided to perform the PVA simulations using the first model, excluding the territorial non-breeding females from the breeding pool. Interestingly, we found that had the immigration flow stopped in the year 2000 with an adult survival of 0.88, the current population would have comprised only five pairs as opposed to 20 (Supplementary Fig. 3.S4). Moreover, even if adult survival had been as high as 0.99, without immigration the predicted population size would have remained lower than observed (Supplementary Fig. 3.S4). Nevertheless, we found that the model including immigration presented a poor fit in the early years (2000-2005) and it improved afterwards (2005-2015, Fig 3.2); contrary, the model excluding immigration presented a better fit in the early years (Supplementary Fig. 3.S4).

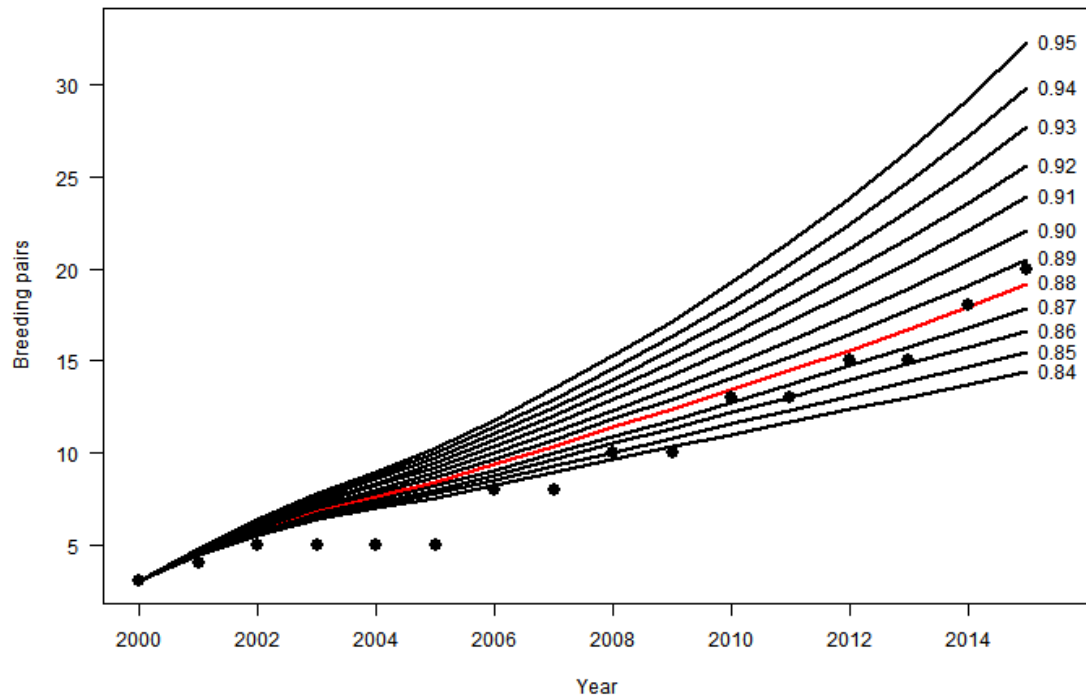


Fig 3.2: Mean number of projected breeding pairs of cranes in the UK under different scenarios of adult survival (0.84-0.95) and fix immigration of one female immigrant per year. Red line indicates the adult survival with the highest likelihood. Black dots represent the observed number of breeding pairs.

3.4.2 *Population viability analysis under different immigration and reintroduction scenarios*

We found a positive mean growth rate for all four simulated scenarios, including and excluding the reintroduced birds and with one and zero female immigrant per year (Fig 3.3). When including the reintroduced birds, the 50-year projections estimated 275 and 195 breeding pairs, for one and zero female immigrant per year, respectively (Fig 3.3); thus cessation of immigration would result in a 29% reduction in the 2065 population size. When excluding the reintroduced birds, the 50-year projection estimated 178 breeding pairs when including one female immigrant per year and 101 when excluding immigration.

In these cases cessation of immigration would result in the 2065 population size being 43% lower (Fig 3.3). In addition, if the current migratory flow is maintained over time, the reintroduction of the 90 juvenile birds into the population will result in a 54% increase in the population size in 50 years' time. The probability of reaching 300 pairs and improving the conservation status in the UK was zero (closed population) and 0.01 (open population) when the reintroduced birds were not included. However in the models that took account of the reintroduction, the probability of reaching favourable conservation status increased to 0.05 and 0.32, assuming a closed or open population, respectively (Fig 3.4).

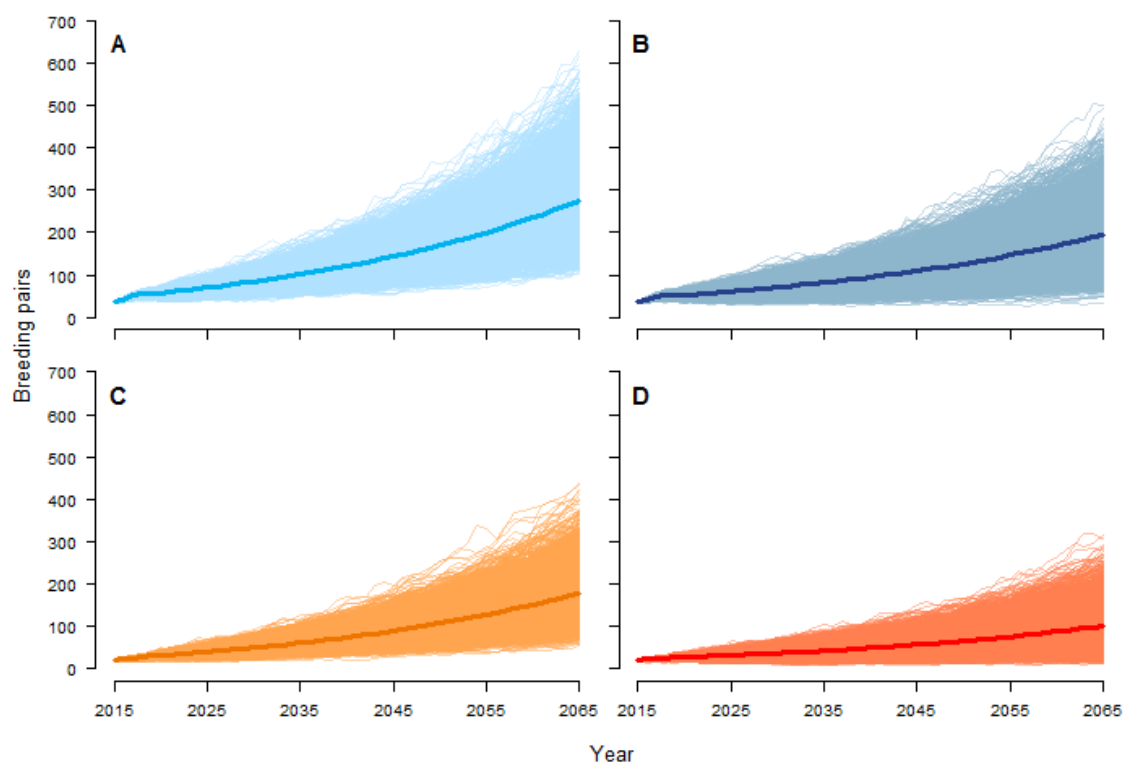


Fig 3.3: Projection of the population trend under four different scenarios: (A) including the released birds and immigration, (B) including the released birds and assuming a closed population, (C) excluding the released birds and assuming immigration and (D) excluding the released birds and assuming a closed population.

3.5 Discussion

Using stochastic matrix population models we assess the role of immigration and reinforcement in driving population dynamics in a long-lived bird. Even though immigration has already been identified as a main driver of population persistence in a series of species (Schaub *et al.* 2010; Schaub *et al.* 2012; Schaub, Jakober & Stauber 2013; Tauler *et al.* 2015; Sanz-Aguilar *et al.* 2016; Weegman *et al.* 2016), the potential effect of population reinforcement on source-sink dynamics has remained largely unexplored. In this study we show that that population reinforcement can decrease the dependency of populations on immigration. In addition, the reinforcement has the added value that an increased breeding pool would decrease population instability associated with stochastic birth and death rates in small populations. This is particularly relevant for longed-lived species that present low annual productivity and are sensitive to high adult mortality (Sæther & Bakke 2000).

Our results reveal that even though the crane population in the UK is self-sustaining in all modelled scenarios and adult survival is high, the population has been acting as a pseudosink with immigration playing a major role in the population persistence and increase (Fig 3.3). However, our results indicate that reinforcing the population with additional birds will have a substantial impact on the species recovery; there is an increase in the projected population size of over 50% from 178 breeding pairs to 275 over the next 50 years when including the reintroduced birds (Fig 3.3). In addition, the dependency on immigration has been reduced after the reintroduction from a 43% to a 29% contribution on the

population growth rate. Moreover, after the population reinforcement, the probability of the population improving its conservation status has increased from just above zero to 32% (Fig 3.4).

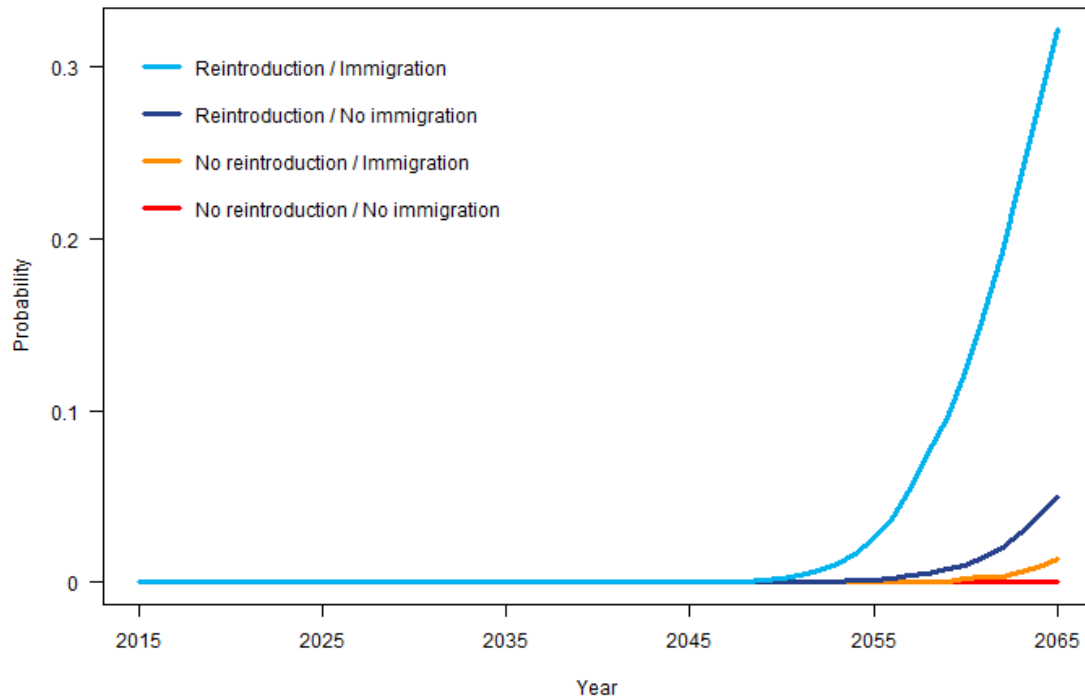


Fig 3.4: Probability of the breeding population reaching 300 pairs and improving its conservation status according to the Red List for Birds under the four simulated scenarios.

Importantly, our models suggest that if the immigration flow had stopped in the year 2000 the population in 2015 would only have had five breeding pairs as opposed to 20 (Supplementary Fig. 3.S4). This relatively high influx of immigrants in recent years contrasts with the population dynamics from 1981 to 2000 where immigration was lower, and the population increased marginally from one to three pairs in 19 years. This pattern was still visible from 2000 to 2005; during those years the model including immigration presented a worse fit than the model excluding it (Fig 3.2 & Supplementary Fig. 3.S4). The lack of immigration into the population could explain the PVA results predicting a mean time to the UK crane population extinction of 17 years (Mathews & Macdonald

2001); since the study was based on data on crane population numbers from 1979 to 1998 (Mathews & Macdonald 2001). Thus, the persistence of the crane population in the UK was probably linked to the increase in immigration rates. This change on immigration could be explained by increasing crane population sizes in western continental Europe, from around 45,000 individuals in 1985 to 300,000 in 2012 (Prange 2012). This six-fold increase occurred in both breeding and wintering areas (Prange 2012). Notably, the number of cranes spending the summer in Sweden rose from 30,000 individuals in 1980 to 100,000 individuals in 2010 (Lundgren 2010), while the number of autumn staging cranes in Germany increased from 45,000 in 1987 to 225,000 in 2008, with the steepest increments from 1995 onwards (Mewes, Prange & Nowald 2010). Such an increment is linked to a series of management strategies, some of which are: the legal protection of the species, the creation and restoration of wetlands, and the protection of key staging, roosting and wintering areas (Meine & Archibald 1996; Deinet *et al.* 2013; Hansbauer 2012).

The crane population in the UK is already self-sustaining thus the reinforcement of the population is likely to have an effect on the population future regardless of additional conservation measures. However, it is worth noting that even if the population trend is positive and the reinforcement of the population could achieve a boost in the population numbers, additional conservation measures are desirable because the recovery of the crane population in the UK is at a very early stage. Adult survival rate of cranes in the UK is relatively high (0.88), so there is likely little scope for improvement. But, the productivity of the population is low (0.56) when compared with other populations in continental Europe, where an average of 0.9 juveniles per breeding pair are fledged every year (Mewes, Prange & Nowald 2010). In order to improve this, crane-friendly

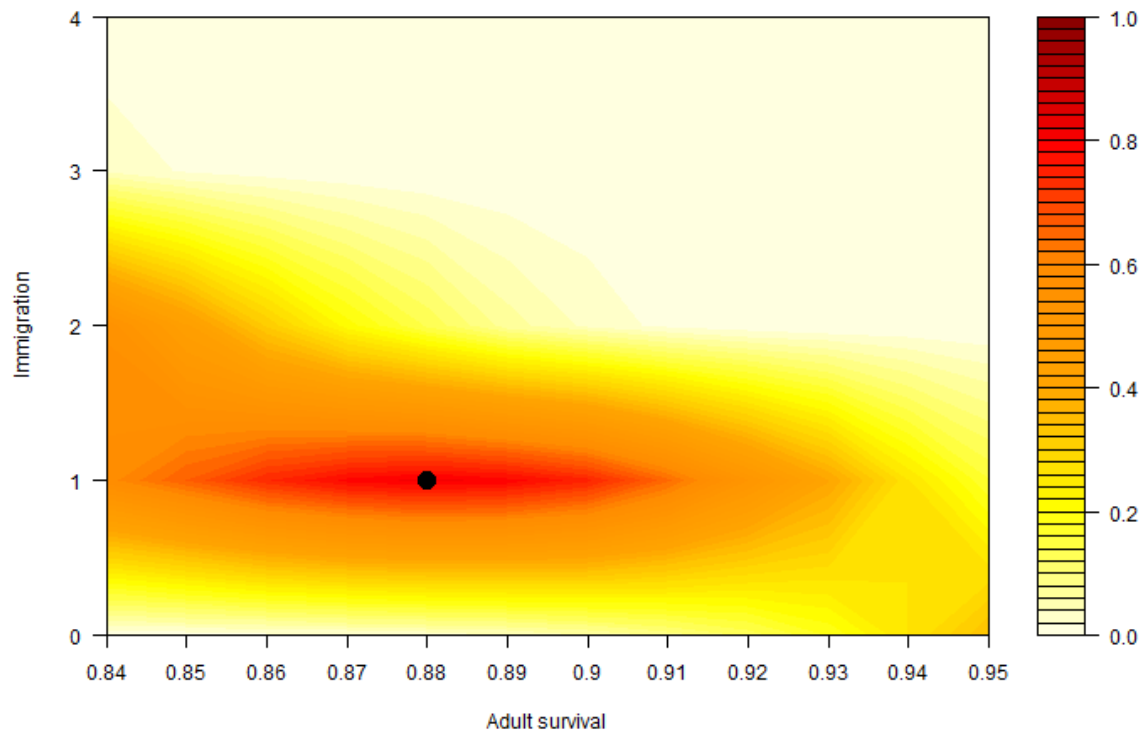
management of water levels should be a priority, since drops in water level during the breeding season can make the eggs or the young chicks vulnerable to some terrestrial predators (Bellebaum & Bock 2009; Prowse 2010; Stanbury & Sills 2012). Conversely, increased water levels can damage nests as nest mounds are usually situated in shallow marsh areas few centimetres above the water level (Stanbury & Sills 2012). Nevertheless, even if management approaches improved the productivity of the population, it would take some time to have an effect on population growth in the long term due to the small clutch sizes and the slow recruitment of the species (Sæther & Bakke 2000).

Reinforcement is becoming a common strategy to boost the size of small populations and avoid stochastic processes that could lead to extinction (Seddon 2010). However it has usually been applied in declining populations that are close to extinction (Cade & Jones 1993; Jones *et al.* 1995; Keedwell, Maloney & Murray 2002; Britt *et al.* 2004; Bretagnolle & Inchausti 2005; Betz, Scheuerer & Reisch 2013). In such cases, conservation actions to reduce threats faced by the population must be implemented beforehand, otherwise restocking the population with additional individuals might delay the population extinction but it will not prevent it (Seddon 2010). Thus, it is critical to understand the causes of population decline before any attempts at restoration (Fischer & Lindenmayer 2000); especially a detailed knowledge of the population demographic processes is necessary, since population decline could be due to either high mortality and low productivity or emigration to other populations (Weegman *et al.* 2016), and the strategies to counteract the population threats would differ.

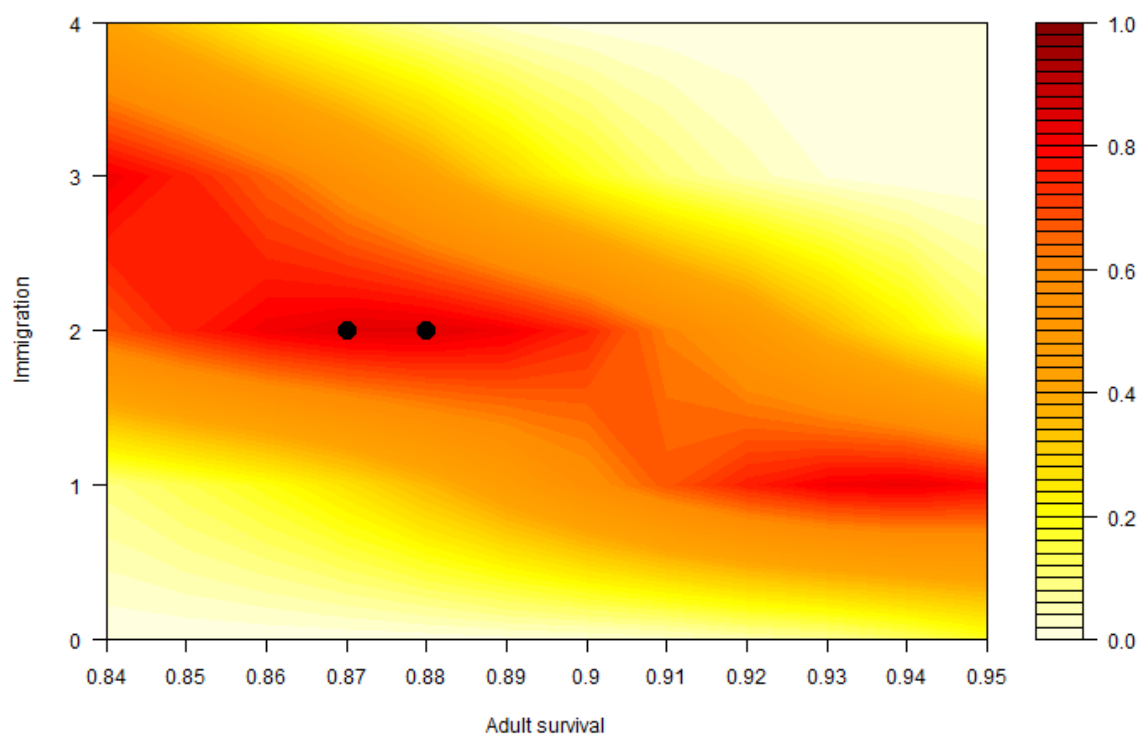
Conservation translocations have been subject to controversy because their high failure rate (Beck *et al.* 1994; Cade & Temple 1995; Fischer & Lindenmayer 2000). However, a recent report by Rewilding Europe about the recovery of several species of mammals and birds across Europe suggests that one of the central management techniques leading to an increase of population numbers was the translocation of individuals to either existing populations or new populations (Deinet *et al.* 2013). In addition, they highlight that the increase in numbers of some species in Western Europe was linked to range expansion from eastern populations to areas where the species had become severely reduced or locally extirpated (Deinet *et al.* 2013). Thus, it seems that the synergetic effect of both immigration from core areas to former range areas and reintroduction and reinforcement techniques are boosting the numbers in several species, such as the European bison (*Bison bonasus*), the alpine ibex (*Capra ibex*), the Eurasian lynx (*Lynx lynx*), the white stork (*Ciconia ciconia*), the peregrine falcon (*Falco peregrinus*) or the red kite (*Milvus milvus*, Deinet *et al.* 2013).

In conclusion, we show that population reinforcement is an effective technique to boost numbers in small and slowly growing populations. In our case, a modest reintroduction programme could result in a 50% increase in the projected population size and a reduction in the long-term dependency on immigration. In addition, in light of the recent increase in the number of animal reintroduction programs worldwide (Seddon, Armstrong & Maloney 2007), we recommend that population dynamics are considered when management strategies, such as reintroductions and reinforcements, are planned.

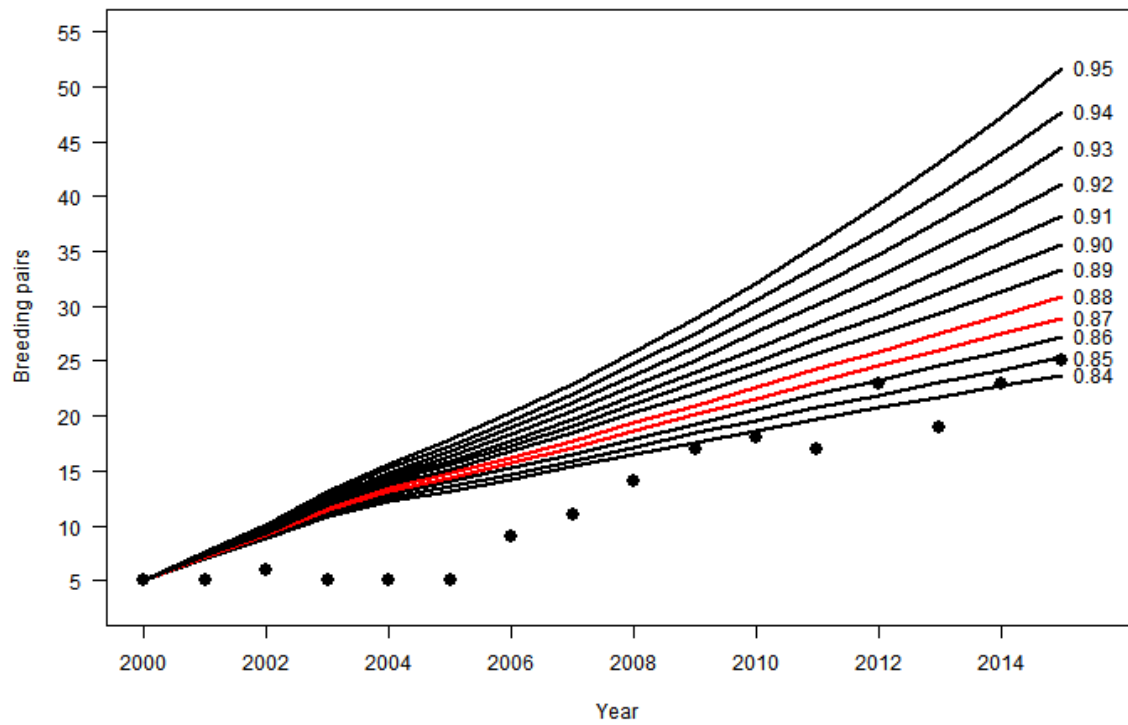
3.6 Supporting Information



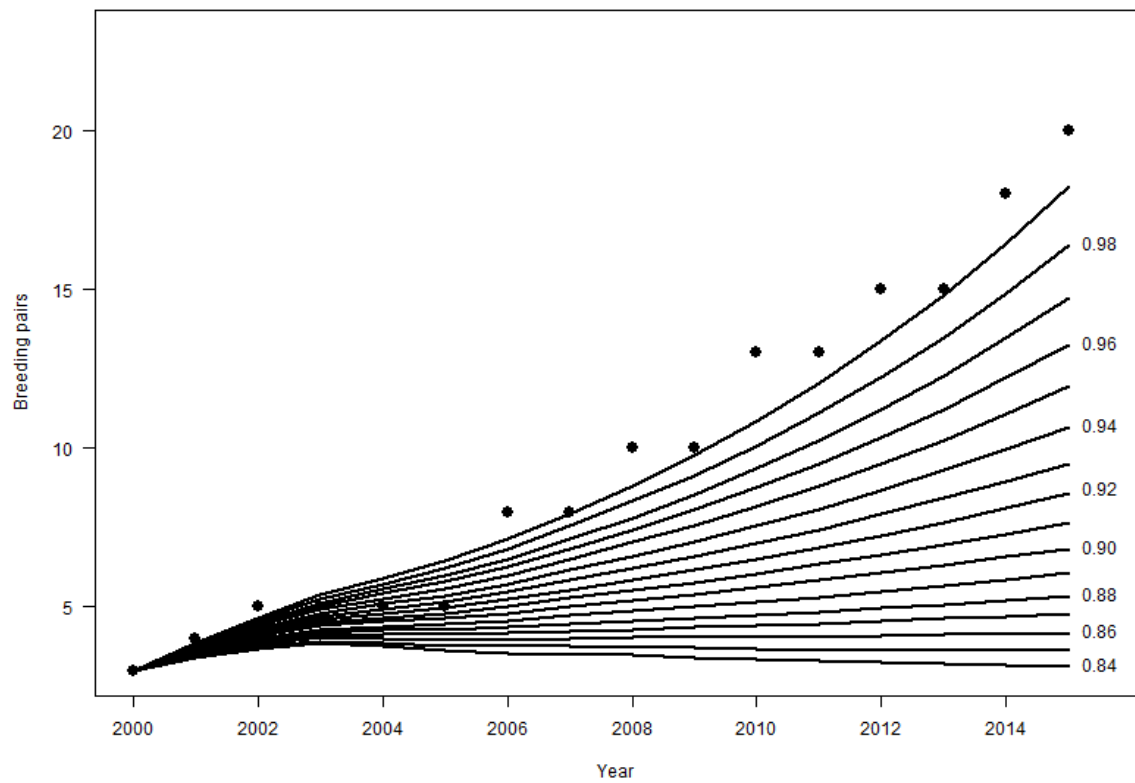
Supplementary Fig 3.S1: Likelihood estimate for a range of values of both adult survival and number of immigrants of cranes in the UK, when sub-adult survival is reduced by 0.2. Black dot indicates the combination with the highest likelihood, 0.88 adult survival and one female immigrant per year.



Supplementary Fig 3.S2: Likelihood estimate for a range of values of both adult survival and number of immigrants of cranes in the UK, when non-breeding females are included in the breeding pool. Black dots indicate the combination with the highest likelihood, 0.88 adult survival and two female immigrants per year.



Supplementary Fig 3.S3: Mean number of projected breeding pairs of cranes in the UK, when non-breeding females are included in the breeding pool, under different scenarios of adult survival (0.84-0.95) and fix immigration of two female immigrants per year. Red lines indicate the adult survival with the highest likelihood. Black dots represent the observed number of breeding pairs.



Supplementary Fig 3.S4: Mean number of projected breeding pairs of cranes in the UK, under different scenarios of adult survival (0.84-0.99) and no immigration. Black dots represent the observed number of breeding pairs.

Chapter 4

Estimating species distribution in dynamic populations using point process models: a case study in the Eurasian crane and perspectives in ecology

In preparation

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4.1 Abstract

1. Understanding and predicting a species' distribution across a landscape is of central importance in ecology, biogeography and conservation biology. However, it presents daunting challenges when populations are highly dynamic (i.e. increasing or decreasing their ranges), particularly for small populations where information about ecology and life history traits is lacking.
2. Phenomenological species distribution models (SDMs) and hybrid models are the usual techniques to estimate distributions of species in relation to their environment. However, those models require that either the species is in equilibrium with their environment or information on key life-history traits.
3. To overcome those limitations, in this study we propose a new approach, spatio-temporal log-Gaussian Cox process models that explicitly model the spatial patterning formed by individuals in space and time relative to covariates and an underlying unobserved continuous process. We aim to predict the future distribution of a recently established population of Eurasian cranes (*Grus grus*) in England and estimate the effect of a reintroduction in the range expansion of the population.
4. Our models show that wetland extent and perimeter-to-area ratio have a positive and negative effect, respectively, in crane colonization probability. Moreover, we also found that cranes are more likely to colonize areas near already occupied wetlands and that the colonization process is progressing extremely slowly. Finally, the reintroduction of cranes in SW England can be considered a human-assisted long-

distance dispersal event and has increased the dispersal potential of the species along an east-west axis in the south of England.

5. Spatio-temporal log-Gaussian Cox process models offer an excellent opportunity for the study of species where information on life history traits is lacking, since births, deaths and dispersal events are represented by the appearance and disappearance of points or the change on the points attributes over time.

4.2 Introduction

Understanding and predicting a species' distribution across a landscape is of central importance in ecology, biogeography and conservation biology (Elith & Leathwick 2009). Species' distribution is a dynamic process, as population ranges change in response to both extrinsic and intrinsic factors (Mooney & Cleland 2001; Lenoir *et al.* 2008; Moritz *et al.* 2008; Mantyka-pringle, Martin & Rhodes 2012; Haddad *et al.* 2015). Climate change, habitat loss, human disturbance or the introduction of non-native species are well-known causes of shifts and reductions in population distribution ranges (Mooney & Cleland 2001; Lenoir *et al.* 2008; Moritz *et al.* 2008; Mantyka-pringle, Martin & Rhodes 2012; Haddad *et al.* 2015). Likewise, efforts to restore original habitats, enhance natural resources and reduce human threats have resulted in the range expansion of several species of vertebrates (Deinet *et al.* 2013; Tauler *et al.* 2015; Sun *et al.* 2016). Nevertheless, the expansion of those populations is highly influenced by intrinsic factors; life history traits, such as intrinsic growth rate or dispersal ability, play a major role in shaping species occurrence (Hastings *et al.* 2005). For declining or increasing populations predicting their

distribution across space and time is extremely challenging (Elith & Leathwick 2009); especially, for small and/or re-establishing populations where data are usually scarce or even missing.

The most common approach to describe distributions of species is to use phenomenological species distribution models (SDMs) that correlate observations of species occurrences with environmental variables. Phenomenological SDMs rely on the implicit assumption that species are in equilibrium with their environment (i.e. the species is spread throughout all the suitable environmental space, see Guisan & Thuiller 2005; Dormann *et al.* 2012). In the current context, where many species are highly dynamic, i.e. increasing, decreasing or experiencing range shifts, this assumption is unrealistic. To overcome this limitation hybrid models have been developed that combine phenomenological and mechanistic approaches, which capture population dynamics (Gallien *et al.* 2010). Hybrid models have been successfully applied in a range of studies. For example, to predict climate-induced range dynamics for the black grouse (*Lyrurus tetrix*) in the Swiss Alps (Zurell *et al.* 2012), bird-mediated spread of invasive plants across northeastern North America (Merow *et al.* 2011), the invasion of a pest insect (*Adelges tsugae*) native to Asia in eastern North America (Fitzpatrick *et al.* 2012), or the spatio-temporal dynamics of a recovering bird species in China (Sun *et al.* 2016).

Unfortunately, to parameterize the mechanistic component of hybrid models, a detailed understanding of key life history parameters is required, for example, fecundity, mortality, dispersal ability or carrying capacity (Gallien *et al.* 2010; Merow *et al.* 2011; Fitzpatrick *et al.* 2012; Zurell *et al.* 2012; Sun *et al.* 2016).

The limited availability of data to estimate these parameters has prevented the implementation of those models for a greater number of species. Importantly, there is often a correlation between lack of information about a species and conservation imperative (i.e. rare threatened species often are the ones with the least information). In this study we propose an alternative approach that does not require the detailed information associated with SDMs or hybrid approaches. Spatio-temporal log-Gaussian Cox processes model the spatial patterning formed by individuals in space and time relative to covariates and an underlying unobserved continuous process which makes up a latent field (Møller & Waagepetersen 2007; Illian, Sørbye & Rue 2012). Given the latent field, the observations (i.e. the locations of the individuals) are considered to be independent. In this context, key life history parameters, such as birth and death, are represented by the appearance and disappearance of points in the species' distribution (Ovaskainen *et al.* 2014). In addition, the observations can be accompanied by qualitative or quantitative information relative to the observation (e.g. health status or size of individuals) that can be fitted as marks in the model (Illian, Sørbye & Rue 2012; Illian *et al.* 2013). Spatial point process models are becoming increasingly relevant in a range of ecological contexts, such as determining underlying process to support species coexistence in plant communities (Law *et al.* 2009; Brown, Illian & Burslem 2016), assessing the processes that regulate spatial locations of muskoxen (*Ovibos moschatus*) herds in Greenland (Illian *et al.* 2012), or estimating factors influencing the frequency of koala (*Phascolarctos cinereus*) visits to trees (Illian, Sørbye & Rue 2012).

The main objective of this study is to illustrate the opportunities provided by spatio-temporal log-Gaussian Cox process models to study population range

expansion for species where knowledge about key life history traits, such as rates and patterns of dispersal, is limited. Here, we focus on the recolonization process of Eurasian cranes (*Grus grus*, cranes hereafter) in England. Historically, cranes were resident birds in the UK, with presence all year around, but they were extirpated in the 16th century after hunting completely depleted the population (Gurney 1921; Stanbury 2011). After they disappeared, cranes only visited the UK rarely as vagrants (Stanbury 2011). In 1979, more than 400 years after the local extirpation of the breeding population a pair established in a wetland area in the Norfolk Broads, eastern England (52° 44' N, 1° 37' E; Stanbury 2011). Over the following years the population grew slowly, to 13-14 breeding pairs in 2010 (Stanbury 2011). The current distribution in the UK comprises areas in the east of the UK, in Norfolk, Cambridgeshire, Oxfordshire, Yorkshire and Aberdeenshire (Stanbury 2011). To boost the species population in the UK, between 2010 and 2014, 90 juvenile birds were released in the Somerset Levels (51° 2' N, 2° 55' W; 56,650 ha), a wetland area situated in SW England, ca. 250 km away from the naturally established population (Soriano-Redondo *et al.* 2016).

Here, we aim to (i) determine the environmental and anthropogenic variables affecting cranes' breeding distribution; (ii) establish the effect of the colonization dynamics in the species distribution, through the spatial autocorrelation structure (latent field); (iii) assess how the colonization dynamics might change over time, through the temporal variation of the spatial autocorrelation structure; (iv) understand the effect of the reintroduction on the species range expansion; and (v) identify the most suitable wetlands for cranes and the wetlands more likely to be colonized in the near future. To do so, we use a recently developed statistical model fitting method, integrated nested Laplace approximation

(INLA), designed to provide a fast and exact approach to fitting complex latent Gaussian models. We fit a joint spatio-temporal model where the wetland locations are considered to be a realisation of a marked log-Gaussian Cox process with marks indicating presence or absence of cranes. In addition, we consider several environmental variables as covariates (Ledo *et al.* 2016). Thus, in this study, key life history parameters are represented through the model mark by the change over time in the occurrence of cranes in the wetland.

4.3 Methods

4.3.1 Study area and species data

Cranes use a wide variety of wetland habitats for breeding (BirdLife-International 2017). For this reason, we aim to determine the wetland features influencing the species colonization in England. To do so, we mapped the wetlands across England combining information from two sources: the UK Land Cover Map 2007 (Morton *et al.* 2011) and the Wetland Vision map of current wetlands (Hume 2008). We limited our study to England because the Wetland Vision map of current wetlands does not cover Scotland and Wales (Hume 2008). We only considered wetland areas over 5 ha since the highest nesting densities recorded for the species are around 8 ha/pair (Johnsgard 1983). In addition, since in the UK cranes have been exclusively nesting in lowland wetlands we excluded wetlands situated in the moorland areas (Stanbury & Sills 2012) based on information from Natural England data products (<http://www.natureonthemap.naturalengland.org.uk>).

For each wetland we calculated four different metrics that could affect the habitat suitability: (i) wetland extent, (ii) perimeter-to-area ratio, (iii) proportion of wetland in the surrounding landscape (10km terrestrial buffer around the wetland), (iv) proportion of surrounding landscape (10km terrestrial buffer) that is urbanized, using urban and suburban areas in England mapped in the UK Land Cover Map 2007. We scaled the four metrics by subtracting the mean and dividing the resulting value by the standard deviation.

To determine whether a wetland had been colonized by cranes, we recorded crane nesting locations (from 2000 to 2015) using a range of sources across England: landowners, site-managers, the Rare Breeding Birds Panel (<http://www.rbbp.org.uk/>), and the Scarce Migrant Reports in the journal *British Birds*. We considered that a wetland was occupied by breeding cranes when (i) there was a direct observation of breeding birds or chicks, (ii) egg remains were found in a nest or a young was present in the area, (iii) a crane family was observed or (iv) there was evidence that suggested the presence of a nest or young. From 2013 onwards (the year in which released cranes first bred) we also recorded the nesting locations of the reintroduced birds. Cranes are very recognisable birds and it is improbable that any nesting location was missed. In the few cases where there was uncertainty about the exact location of the nesting site, we assigned it to the closest wetland area.

4.3.2 *Statistical analysis*

To estimate the drivers of crane distribution in England and predict crane occurrence over time, we used INLA and fit marked log-Gaussian Cox process models (i.e. joint models with two likelihoods and two responses) for the wetland locations and the presence of cranes (as a mark). We included the four

scaled environmental variables mentioned above as covariates of the mark. For the responses, we specified two different distributions: a Poisson distribution for the wetland locations and a binomial distribution for the presence of breeding cranes. To predict how the colonization process is likely to change over time we used a first order random walk to model the temporal autocorrelation of the breeding locations (2000-2015). In addition, as we were modelling in a Bayesian context, we used normal uninformative priors (with variance set to 10). We use a stochastic partial differential equation (SPDE) model to account for spatial autocorrelation (Lindgren 2012; Cameletti *et al.* 2013). This method relies on constructing a Delaunay triangulation (mesh) of the domain. This mesh is the structure on which the representation of the field is based (Fig 4.1). To avoid edge effects (i.e. increased variance at borders) in the latent field prediction we extended the mesh beyond the English border. To understand the effect of the reintroduction on the species range expansion we run two main models: the first only comprising the natural population (NAT model hereafter) and the second one adding the nesting locations of the reintroduced birds (NAT-RE model hereafter).

Measures to test the robustness for log-Gaussian Cox models are not well established and remain under development. Therefore, to test the strength of the results we run three additional models: the first including the nesting pairs from the natural population from 2000 to 2010 (note that during that period both datasets, including and excluding the reintroduced birds, are identical), the second one including the nesting pairs from the natural population from 2011 to 2015 and the third one including the nesting pairs from the natural population and the reintroduced population from 2011 to 2015.

Finally, we determined the 20 most likely areas to be colonised using the two models (NAT and NAT-RE). For each model we also calculated two scenarios for the predicted values, the first using only the estimates of the significant covariates and the second one adding the spatial autocorrelation structure in the predicted values.

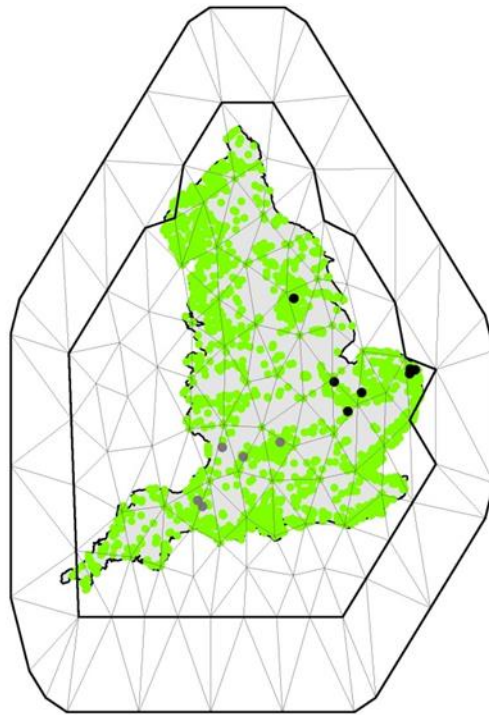


Fig 4.1: Delaunay triangulation (mesh) over the study area. Green dots represent the 2,526 wetlands in England, black dots the wetlands occupied by nesting pairs from the natural population and grey dots the wetlands occupied by nesting pairs from the reintroduced population.

4.4 Results

4.4.1 *Changes in wetland occupancy by breeding pairs*

We mapped a total of 2,526 wetlands across England ranging from 5 to 24,707 ha with perimeter-to-area ratios ranging from 0.002 to 0.077. The proportion of surrounding wetland and the proportion of urbanized areas ranged from 0 to

0.57 and from 0 to 0.81, respectively. When considering the natural population only (NAT dataset), the number of occupied wetlands increased steadily in the 15-year time period, from one wetland in 2000 in the Norfolk Broads to seven in 2015 spread across Norfolk, Cambridgeshire and Yorkshire (Fig 4.1). When including the reintroduced individuals in the dataset (NAT-RE dataset), it only differed from the NAT dataset from 2013 onwards, when the reintroduced birds started nesting in several areas in Somerset, Gloucestershire and Oxfordshire (Fig 4.1). The total number of breeding pairs reached 30 in 2015 spread over 12 wetland areas across England (Fig 4.1).

4.4.2 *Drivers of crane colonization*

In the NAT model, wetland extent positively affected crane colonization probability, i.e. cranes were more likely to settle in larger wetlands (Table 4.1). This is in contrast to the perimeter-to-area ratio, which had a negative effect, i.e. cranes preferred wetlands where edges were minimised (Table 4.1). Our model indicated that the two landscape features, proportion of wetland and urban areas in the surroundings did not affect the cranes' establishment probability (Table 4.1). The results from the NAT-RE model were similar: wetland extent and the perimeter-to-area ratio showed the same pattern (Table 4.2). However, in this instance the proportion of surrounding wetland area had a small negative effect on colonization probability (Table 4.2). The results from the three additional models were similar: wetland extent had a significant positive effect in the two models including only the nesting pairs from the natural population from 2000 to 2010 and from 2011 to 2015 (Supplementary Table 4.S1 & 4.S2), and perimeter-to-area ratio had a significant negative effect in the model including the nesting pairs from the natural population from 2000 to 2010 and the model

including the nesting pairs from the natural population and the reintroduced population from 2011 to 2015 (Supplementary Table 4.S1 & 4.S3). The proportion of surrounding wetland and urban areas had no significant effect in any of the three models. Therefore, we focused on the effect of the wetland extent and the perimeter-to-area ratio in the discussion section.

Table 4.1: Posterior means, standard deviations and 95% credible intervals for the covariates and the spatio-temporal random fields (spatial smoothness, scale and temporal autocorrelation of the latent field, respectively) estimated in the NAT model which only includes the nesting pairs from the natural population.

	Mean	SD	2.5% quant.	97.5% quant.
Wetland extent	1.7793	0.2042	1.3784	2.1798
Perimeter-to-area ratio	-0.9678	0.3575	-1.6697	-0.2664
Proportion of surrounding wetland	-0.6140	0.5329	-1.6604	0.4314
Proportion of surrounding urbanized areas	0.5525	0.5334	-0.4948	1.5990
Log(τ)	-3.9672	0.0335	-4.0386	-3.8480
Log(κ)	-0.5706	0.0213	-0.6164	-0.4842
ρ	0.9998	0.0001	0.9995	1.0000

The spatial autocorrelation structure had a strong influence on the species' distribution, especially when considering only the natural population (Fig 4.2A). Nevertheless, the spatial autocorrelation structure had a substantial uncertainty, which increased with the distance from the colonized wetlands (Fig 4.2B). However, when the reintroduced birds were accounted for, the intensity of the spatial autocorrelation structure decreased (Fig 4.2C). Likewise, the uncertainty was lower, although there was increased uncertainty further away from the colonized wetlands (Fig 4.2D). Thus, the weight of the spatial autocorrelation

structure relative to the covariates was higher in NAT dataset models than in NAT-RE models. We also found an extremely high temporal autocorrelation ($\rho > 0.99$ in both models) in the spatial autocorrelation structure from one year to the next, implying that the spatial structure is unlikely to change in the near future, which also means that the colonization process is progressing extremely slowly (Tables 4.1 & 4.2).

Table 4.2: Posterior means, standard deviations and 95% credible intervals for the covariates and the spatio-temporal random fields (spatial smoothness, scale and temporal autocorrelation of the latent field, respectively) estimated in the NAT-RE model which includes the nesting pairs from the natural population and the reintroduced population.

	Mean	SD	2.5% quant.	97.5% quant.
Wetland extent	0.1234	0.0494	0.0263	0.2203
Perimeter-to-area ratio	-3.1559	0.3486	-3.8403	-2.4721
Proportion of surrounding wetland	-0.6920	0.3377	-1.3550	-0.0296
Proportion of surrounding urbanized areas	0.0402	0.3966	-0.7385	0.8183
Log(τ)	-3.5466	0.4247	-4.4008	-2.7320
Log(κ)	-0.4607	0.2433	-0.9746	-0.0249
ρ	0.9992	0.0007	0.9973	0.9999

With respect to the NAT model, when accounting for the spatial autocorrelation and the significant covariates, we found that the 20 wetlands most likely to be colonized were in close proximity to the currently occupied wetlands in eastern England (Fig 4.3A). However, when we ignored the spatial autocorrelation and simply determined the more suitable wetlands for cranes according with our covariates, we found that they were spread across much of England (Fig 4.3B).

The NAT-RE model followed a similar pattern: when considering the spatial autocorrelation structure and the significant variables, the most likely areas to be colonized were near to the already occupied wetlands (Fig 4.3C). Likewise, the more suitable wetlands in the NAT-RE model were spread across England (Fig 4.3D). Moreover, the site where cranes were reintroduced in the Somerset Levels obtained the third highest score using the NAT model estimates. It is also encouraging to note that the reintroduced cranes have nested in two of the top 20 wetlands predicted by the NAT model (Fig 4.3B). Of the top 20 wetlands that each model highlighted, seven were selected by both NAT and NAT-RE models.

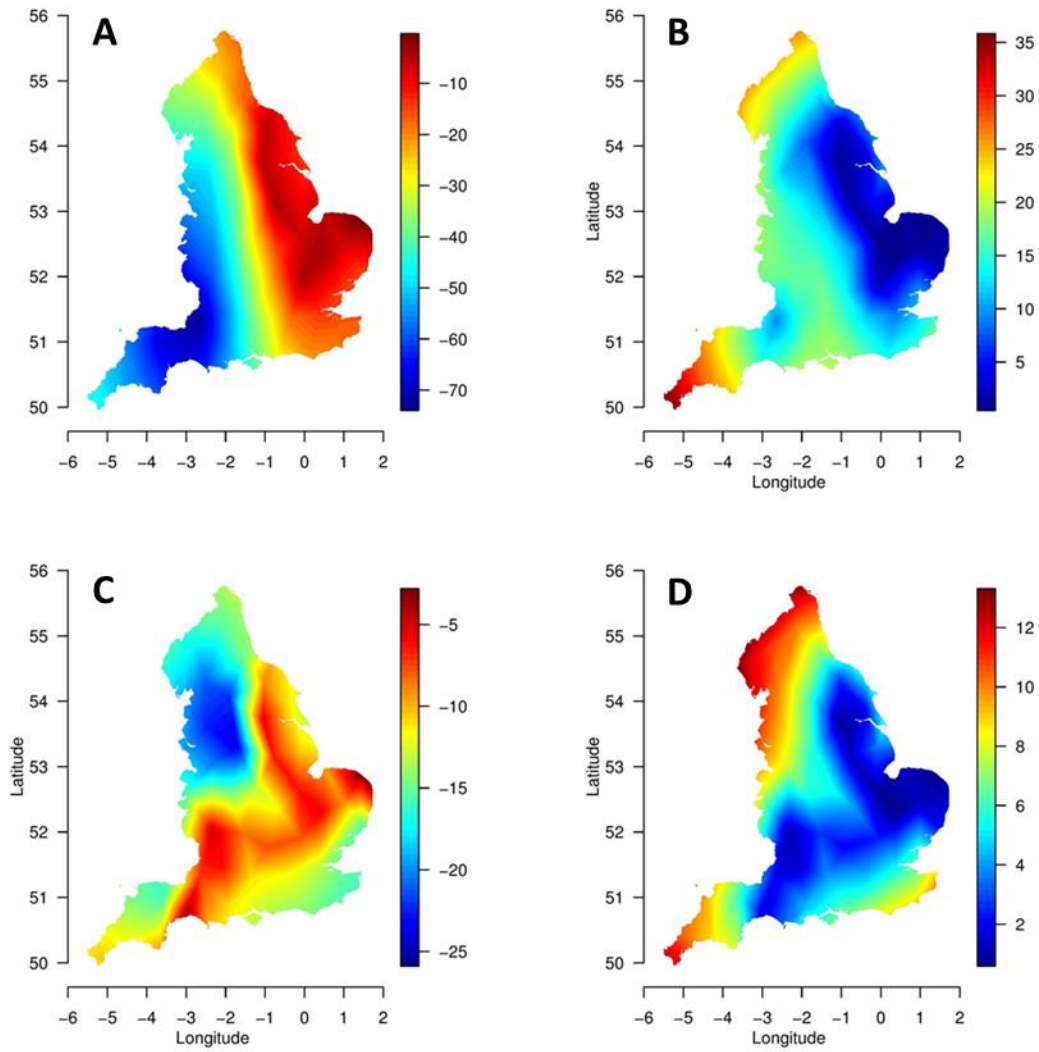


Fig 4.2: The (A) posterior mean and (B) the posterior standard deviation of the spatial autocorrelation structure estimated in the NAT model which only includes the nesting pairs from the natural population; and the (C) posterior mean and (D) the posterior standard deviation of the spatial autocorrelation structure estimated in the NAT-RE model which includes the nesting pairs from the natural population and the reintroduced population. Once accounted for the effect of the covariates, given the spatial autocorrelation structure, the observations of cranes occurrence are considered to be independent. The spatial autocorrelation structure accounts for the colonization dynamics. The higher values in the posterior mean indicate a stronger autocorrelation structure.

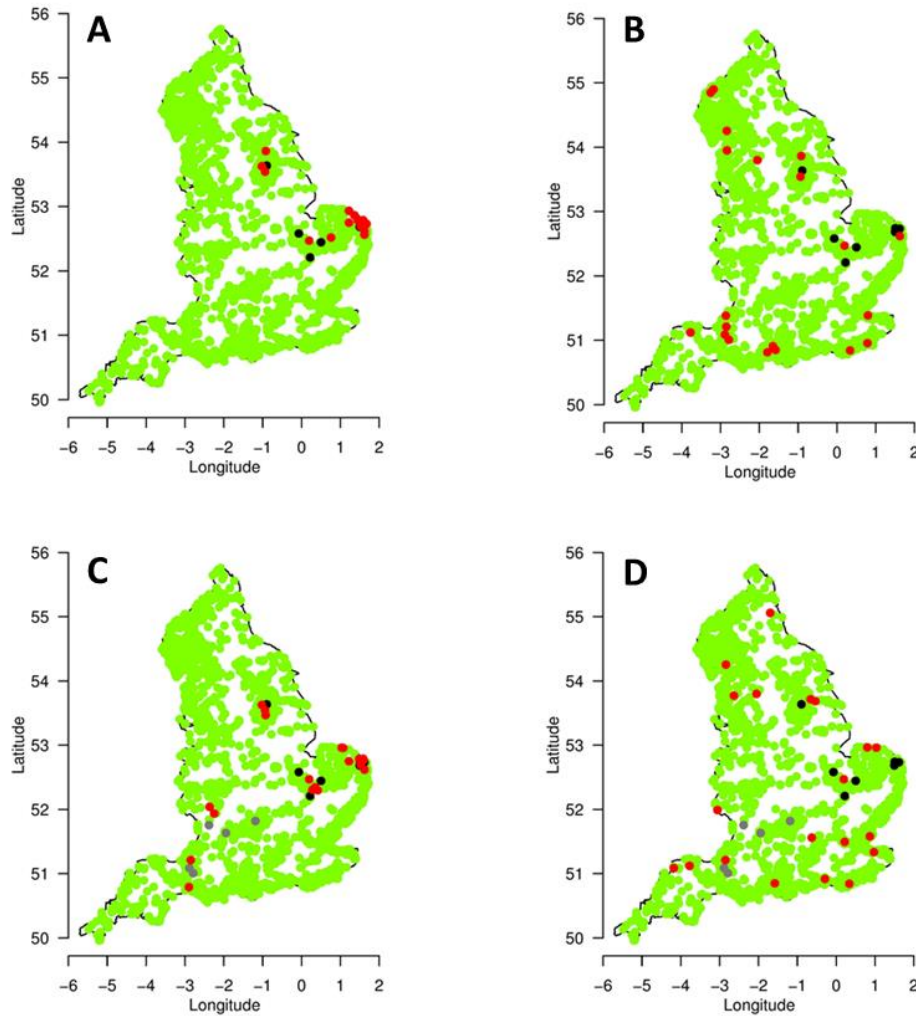


Fig 4.3: Wetlands most likely to be colonised in the near future. Green dots represent the 2,526 wetlands in England, black dots the wetlands occupied by nesting pairs from the natural population and grey dots the wetlands occupied by nesting pairs from the reintroduced population. The red dots represent the 20 most likely wetlands to be colonised, when (A) considering the posterior mean of the covariates and the spatial autocorrelation structure and (B) considering only the posterior mean of the covariates estimated in the NAT model which only includes the nesting pairs from the natural population; and when (C) considering the posterior mean of the covariates and the spatial autocorrelation structure and (D) considering only the posterior mean of the covariates estimated in the NAT-RE model which includes the nesting pairs from the natural population and the reintroduced population.

4.5 Discussion

In this study, for the first time, point process models were applied to gain an understanding of and to predict the recolonization process of a bird species in a dynamic population. Specifically, we used marked log-Gaussian Cox process models to model the spatio-temporal distribution of cranes in England, where the species is recolonizing several wetland areas, through immigration from mainland Europe and a subsequent reintroduction in SW England, after the extirpation of its breeding population in the 16th century. We found that crane colonization is progressing at a very slow rate and that they favour areas near already colonized wetlands. However, within those areas, cranes are selecting large wetlands with particularly low perimeter-to-area ratios. In addition, the crane reintroduction has increased the probability of recolonization in the west of England.

The main advantage of log-Gaussian Cox process models is that they account for spatial structure that remains unexplained by the covariates by using a latent field that can be modelled over time and captures the dynamism of ecological systems. In our case, the spatial autocorrelation structure was a proxy for the colonization process; since cranes started recolonizing England from the east, the occupied wetland tend to be restricted to the eastern part of the country, even though there are apparently suitable wetlands further afield. In ecological terms the factors affecting the spatial structure of the occupied wetlands are likely to be natal philopatry and conspecific attraction (Timoney 1999; Nesbitt, Schwikert & Folk 2002) which would explain why occupied wetlands aggregate in clusters in some areas, such as in the Norfolk Broads and Cambridgeshire Fens, with cranes tending to settle adjacent to areas that are already occupied

(Timoney 1999; Nesbitt, Schwikert & Folk 2002). These phenomena are well captured in the spatial autocorrelation structure, which show a gradient through the east-west axis, with higher values on the eastern side. The high temporal autocorrelation obtained in our models show that the spatial autocorrelation structure should remain constant over time, which indicates that turnover of occupied wetlands between years is low and as such colonization will progress slowly (Table 4.1 & 4.2).

Overall, our models suggest that cranes prefer large wetlands with low perimeter-to-area ratios (i.e. low amounts of edge habitat, Tables 4.1 & 4.2). Nevertheless, the four selected covariates only captured general wetland features; therefore, there could be a number of site-level characteristics relevant for crane colonization that remained unaccounted for. We observe that at a country-level, the spatial autocorrelation structure of the marked point process is more relevant in determining the potential colonization areas than the spatial characteristics of the wetlands, with wetland near to occupied sites having a higher probability of occupancy than wetlands further away (Figs 4.2A & 4.2C). However, at a regional-level, differences in the spatial autocorrelation structure among wetlands are reduced and the importance of the environmental covariates increases significantly. Therefore, assuming constant environmental conditions and the same rate of spread as the previous 15 years, our study indicates that cranes will colonize large wetlands with low perimeter-to-area ratios near to the currently occupied wetlands.

Interestingly, the two covariates that positively affected crane colonization (in both the NAT and the NAT-RE model), large wetland extent and low perimeter-to area ratio, are indicators of high quality habitat and low fragmentation

(Laurance & Yensen 1991). These variables are linked to high species richness, abundance and nest success in several grassland and wetland bird species, such as upland sandpiper (*Bartramia longicauda*), purple heron (*Ardea purpurea*) and little bittern (*Ixobrychus minutus*) (Burger, Burger Jr & Faaborg 1994; Helzer & Jelinski 1999; Winter *et al.* 2006; Benassi, Battisti & Luiselli 2007). Several mechanisms may explain birds' preference for larger wetlands: higher habitat heterogeneity (Golet *et al.* 2001), higher conspecific attraction (Benoit & Askins 2002), longer hydroperiods (Snodgrass *et al.* 2000), adequate territory size requirements (Worrall, Peberdy & Millett 1997) or higher invertebrate food availability (Euliss & Mushet 1999; Schulz 2004; Tarr, Baber & Babbitt 2005). In addition, in England, most of the remaining large wetlands are under protection being commonly designated as Sites of Special Scientific Interest (SSSI, Natural England, <https://designatedsites.naturalengland.org.uk/>) and there is evidence that protected wetlands have been used by colonizing birds as stepping stones to for the permanent population establishment (Hiley *et al.* 2013).

The comparison between the NAT and the NAT-RE model produced some interesting information about the effect of the reintroduction on potential population spread. Our results indicate that, prior to the reintroduction, even though the model predicted a westward spread, the probability of the species reaching the west of England was extremely low, since the spatial autocorrelation structure should remain constant over time (Fig 4.2A). Thus, even large wetlands with low perimeter-to-area ratios scored substantially low colonization probabilities compared to less suitable sites in eastern England. Our ability to predict the likelihood of colonization (Fig 4.2B) in wetlands further away from the core colonized areas is hindered by the uncertainty associated to

the spatial autocorrelation structure. However, after the reintroduction, the potential colonization areas spread along an east-west axis in the south of England (Fig 4.2C). Remarkably, the spatial autocorrelation decreased dramatically in the RE-NAT model (Figs 4.2A & 4.2C), implying that the importance of relevant covariates (the habitat) in the colonization process increased, which has relevant ecological implications: reintroduced cranes are tending to colonize wetlands more as a function of habitat suitability than proximity to already occupied wetlands. This increase in dispersal might be due to the particular aspects of the reintroduction. For example, large numbers of birds reaching maturity the same year, in contrast to the natural population where the process is gradual, could have increased competition for suitable breeding territories locally which forced the pairs to disperse further away; and/or the effect of the natal philopatry could have been reduced because the reintroduced birds were hand-reared in enclosed facilities. Thus, even though our results suggest areas far away from the current core range should remain unoccupied, the reintroduction could be seen as human-assisted long-distance dispersal event that would promote the population spread westward. Moreover, there is already evidence of connectivity between the natural and the reintroduced population, with a pair formed by one individual from each population nesting in 2015 (Soriano-Redondo *et al.* unpublished).

To date, log-Gaussian Cox process models have been applied in several areas of research, such as meteorology or policy (Illian, Sørbye & Rue 2012; Illian *et al.* 2013; Cosandey-Godin *et al.* 2014; Gómez-Rubio, Cameletti & Finazzi 2015; Python *et al.* 2016). However, to our knowledge, only one study in ecology has used spatio-temporal log-Gaussian Cox process models and this modelled the pattern of shark bycatch through time and space in the Canadian Arctic

(Cosandey-Godin *et al.* 2014), rather than actual geographic distributions. Thus, this study provides a new approach to modelling spatial change in dynamic populations. Although we focus on a population that is expanding its range, this methodology can be used when population ranges are contracting or shifting and it could be particularly relevant for the study and conservation of rare species under climate change, where information on life history traits is lacking and management strategies are time sensitive. Spatio-temporal log-Gaussian Cox process models could help predict the spatial change of population distribution through the modelling of the latent field even when the underlying causes for that change are only partially understood. Another field that could benefit from this approach is the study of biological invasion, since the study of particular invasion events is usually challenging due to small sample sizes and the influence of local factors (Guisan *et al.* 2014).

4.6 Supporting Information

Supplementary Table 4.S1: Posterior means, standard deviations and 95% credible intervals for the covariates and the spatio-temporal random fields (spatial smoothness, scale and temporal autocorrelation of the latent field, respectively) estimated in the model that only model which only includes the nesting pairs from the natural population from 2000 to 2010. Note that the time frame is prior to the reintroduction, thus, both datasets (including and excluding the reintroduced birds) are identical.

	Mean	SD	2.5% quant.	97.5% quant.
Wetland extent	2.8893	0.4044	2.0953	3.6826
Perimeter-to-area ratio	-0.3122	0.4757	-1.2461	0.6209
Proportion of surrounding wetland	-0.3098	0.8837	-2.0448	1.4238
Proportion of surrounding urbanized areas	0.0876	1.0422	-1.9586	2.1321
Log(τ)	-4.5057	0.0015	-4.5088	-4.5030
Log(κ)	-0.7301	0.0036	-0.7382	-0.7243
ρ	0.9999	0.0000	0.9999	0.9999

Supplementary Table 4.S2: Posterior means, standard deviations and 95% credible intervals for the covariates and the spatio-temporal random fields (spatial smoothness, scale and temporal autocorrelation of the latent field, respectively) estimated in the model that only model which only includes the nesting pairs from the natural population from 2011 to 2015.

	Mean	SD	2.5% quant.	97.5% quant.
Wetland extent	1.3886	0.2448	0.9079	1.8689
Perimeter-to-area ratio	-1.5243	0.5459	-2.5961	-0.4534
Proportion of surrounding wetland	-0.7021	0.7209	-2.1174	0.7121
Proportion of surrounding urbanized areas	0.2871	0.6772	-1.0424	1.6155
Log(τ)	-3.4916	0.3056	-4.1058	-2.9064
Log(κ)	-0.9536	0.2908	-1.5198	-0.3765
ρ	0.9996	0.0006	0.9980	1.0000

Supplementary Table 4.S3: Posterior means, standard deviations and 95% credible intervals for the covariates and the spatio-temporal random fields (spatial smoothness, scale and temporal autocorrelation of the latent field, respectively) estimated in the model that only model which includes the nesting pairs from the natural population and the reintroduced population from 2011 to 2015.

	Mean	SD	2.5% quant.	97.5% quant.
Wetland extent	0.0534	0.0390	-0.0232	0.1300
Perimeter-to-area ratio	-3.3142	0.4766	-4.2500	-2.3792
Proportion of surrounding wetland	-0.2131	0.3333	-0.8675	0.4407
Proportion of surrounding urbanized areas	-0.2905	0.4206	-1.1164	0.5347
Log(τ)	-2.6245	0.0342	-2.6961	-2.5622
Log(κ)	-0.8003	0.0724	-0.9190	-0.6394
ρ	0.9915	0.0006	0.9904	0.9927

Chapter 5

Ecological Responses to Extreme Flooding Events: A Case Study with a Reintroduced Bird

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5.1 Abstract

In recent years numerous studies have documented the effects of a changing climate on the world's biodiversity. Although extreme weather events are predicted to increase in frequency and intensity and are challenging to organisms, there are few quantitative observations on the survival, behaviour and energy expenditure of animals during such events. We provide the first data on activity and energy expenditure of birds, Eurasian cranes *Grus grus*, during the winter of 2013–14, which saw the most severe floods in SW England in over 200 years. We fitted 23 cranes with telemetry devices and used remote sensing data to model flood dynamics during three consecutive winters (2012–2015). Our results show that during the acute phase of the 2013–14 floods, potential feeding areas decreased dramatically and cranes restricted their activity to a small partially unflooded area. They also increased energy expenditure (+15%) as they increased their foraging activity and reduced resting time. Survival did not decline in 2013–14, indicating that even though extreme climatic events strongly affected time-energy budgets, behavioural plasticity alleviated any potential impact on fitness. However under climate change scenarios such challenges may not be sustainable over longer periods and potentially could increase species vulnerability.

5.2 Introduction

There is ample evidence that climate change has important consequences for biota and ecosystems (Walther *et al.* 2002; Mair *et al.* 2012; Merilä 2012). A number of studies have shown that ecological and evolutionary responses to global warming are widespread and range from genetically-based adaptations,

to population-level effects and geographic range shifts, as well as ecosystem-level reorganisation (Beaugrand *et al.* 2002; Walther *et al.* 2002; Réale *et al.* 2003; Levitan & Etges 2005; Duriez *et al.* 2012). Moreover, climatic change is predicted to have other consequences than simply shifting average values. In particular it is expected to result in an escalation of extreme weather events (Rahmstorf & Coumou 2011; Coumou & Rahmstorf 2012). However, research into the impact of extreme weather on biota presents daunting challenges: such events are rare, brief and difficult to predict (Jentsch, Kreyling & Beierkuhnlein 2007). Nevertheless, there is some evidence indicating that these short-term episodes can have long-term ecological consequences and have the potential to completely transform ecosystems (Gutschick & Bassirirad 2003; Maron *et al.* 2015). They can lead to drastic reductions in population size of some species (Altwegg *et al.* 2006; Viera *et al.* 2006; Frederiksen *et al.* 2008; Deville *et al.* 2014), and in some instances extinctions have been associated with extreme weather events (Parmesan, Root & Willig 2000). For example, several populations of Edith's Checkerspot butterfly (*Euphydryas editha*) disappeared after extreme fluctuations in precipitation in California, USA, and it is known that some animal populations experience increased mortality during extreme winter conditions (Singer & Thomas 1996; McLaughlin *et al.* 2002). But some outcomes are much more indirect, opaque and difficult to predict, such as the impact of a single flood event upon a desert rodent community in Arizona, USA. This caused differential species-specific mortality and reset long-term population trends, leading to a complete rearrangement of the community, resulting in the displacement of a native by an invasive species (Thibault & Brown 2008).

These population and community changes are a consequence of the sum of individual responses to a variable environment (Benton, Plaistow & Coulson 2006; Charmantier *et al.* 2008) and most research on the effects of extreme weather events thus far has focused on population-level responses, neglecting the mechanisms that might underlie these population dynamics (Benton, Plaistow & Coulson 2006). There is evidence to suggest that different individuals exhibit different physiological, morphological and behavioural adjustments to climatic challenges (Root 1994; Parmesan, Root & Willig 2000; Charmantier *et al.* 2008; Nowack *et al.* 2015; Senner *et al.* 2015), and that individuals in better body condition are better able to cope than individuals that were struggling beforehand (Wingfield, Kelley & Angelier 2011). In such instances, understanding the causes and consequences of individual variation in response to extreme weather events is required to better predict downstream demographic and population level consequences under different scenarios.

In the UK, evidence suggests that winter cyclones are becoming more common and their intensity is increasing (Wang *et al.* 2013). Additionally, there are strong indications that the distribution of daily precipitation is altering, becoming more intense in winter and less intense in summer (Osborn *et al.* 2000), and the frequency of daily heavy rain events has increased from a 1 in 125 day event in the 1960 s to 1 a in 85 day event in 2009 (Osborn *et al.* 2000; Jones *et al.* 2013). Some models also suggest that one-hour precipitation extremes could increase by ca. 14% per degree of warming in most parts of Europe (Lenderink & van Meijgaard 2008). Thus, there is likely to be an increase in the frequency and intensity of extreme winter precipitation in Europe. This in turn will lead to an increase in flooding events, and how organisms might respond to these extremes remains unknown.

In this study, we present data on the response to an extreme flooding event by Eurasian cranes (*Grus grus*; hereafter cranes), which were reintroduced in SW England from 2010 to 2014. Cranes are terrestrial feeders that rely on unflooded areas or shallow waters when feeding. Thus, a reduction of available foraging area because of extreme floods has the potential to impact fitness (Archibald & Meine 1983). We monitored this crane population over three consecutive winters (2012–13, 2013–14 and 2014–15) and deployed GPS and accelerometry devices on multiple individuals (see methods for further details) to estimate their distribution, behaviour and energy expenditure. We performed monthly surveys of the population to determine survival and reproductive success. We also gathered data from satellite images and water gauges in the region to estimate flooding extent and dynamics. During winter 2013–14, the south of England experienced exceptional winter rainfalls, which led to the most extensive floods since the 19th Century (Slingo *et al.* 2014). Here we use these data to assess the impact that this extreme weather event had on the individuals of the population, relative to the previous and the following year, in order to gain a better understanding of the mechanisms by which organisms cope with these extraordinary events.

5.3 Results

5.3.1 *Fieldwork outcome*

To estimate the response of birds to winter floods we focused our research on the months between November and March for three consecutive winters (2012–2015). We obtained telemetry locations from: 12 birds for the first winter (2012–13); 9 birds for the second winter (2013–14); and 7 birds for the last winter

(2014–15). Five of the birds provided data in both the first and the second winter. We also recovered acceleration data from 7 birds in the first winter; from 4 birds in the second; and from 5 birds in the third winter. In this case, 2 birds provided data in both the first and the second winter. We did not use acceleration data from the third winter as the tags had different characteristics, with higher sensitivity, producing acceleration patterns that were not comparable with data from previous winters.

5.3.2 *Survival and reproduction*

Monthly resightings showed that during the first winter (2012–13) the survival was 98.1% (1 crane out of 52 died), in the second winter (2013–14), when the extreme flooding event occurred, survival was 94.1% (4 cranes out of 68 died) while in the third winter survival was 96.2% (3 cranes out of 77 died). These overwinter survival estimates are generally higher than rates ($\approx 90\%$) reported in studies from other locations (Johnsgard 1983). All birds in this study were young; released as juveniles in the summers of 2010–2014. Thus in 2012–13, birds were in their first to third years, in their first to fourth years during 2013–14, and in their first to fifth years during 2014–15. The analysis showed that there were no significant differences in survival rates among the three winters ($\chi^2 = 1.2$, $df = 2$, $P = 0.55$). We were not able to test the effects of floods on subsequent breeding success because of the small number of nesting attempts among a population of immature birds; in 2013 there were none; in 2014 there were two unsuccessful breeding attempts; and in 2015 three pairs nested in the area, but only two chicks from one pair fledged.

5.3.3 *Flooding estimates*

The extent of flooding in the Somerset Levels varied greatly across the three years of study, with the second winter having the most extensive floods (Fig. 5.1A). The flood dynamics also varied across years; in the first winter the peak of the flood was reached rapidly, in the second winter the flood started later and had a slower rate of increase until it reached the highest peak and in the third winter there was only a small and short flood (Fig. 5.1A). To quantify the flood extent, we downloaded 19 Landsat images spanning the duration of the study. Of these, 10 were cloud-free and met the necessary requirements for measuring the extent of flooding. We also obtained daily water gauge measurements from the study area to allow closer examination of flood progression. The correlation between flood extents estimated from the images and water gauge measurements was very strong and highly significant (linear regression, $P < 0.001$, $R^2 = 0.9$, Fig. 5.1B). We then used this relationship to estimate daily flood extent from water gauge measurements across the three winters. These estimates revealed that, in the second winter, at the peak of the extreme flooding event, the area of the flood was 12% and 28% greater than at the highest point of the floods in the previous year and in the subsequent year, respectively.

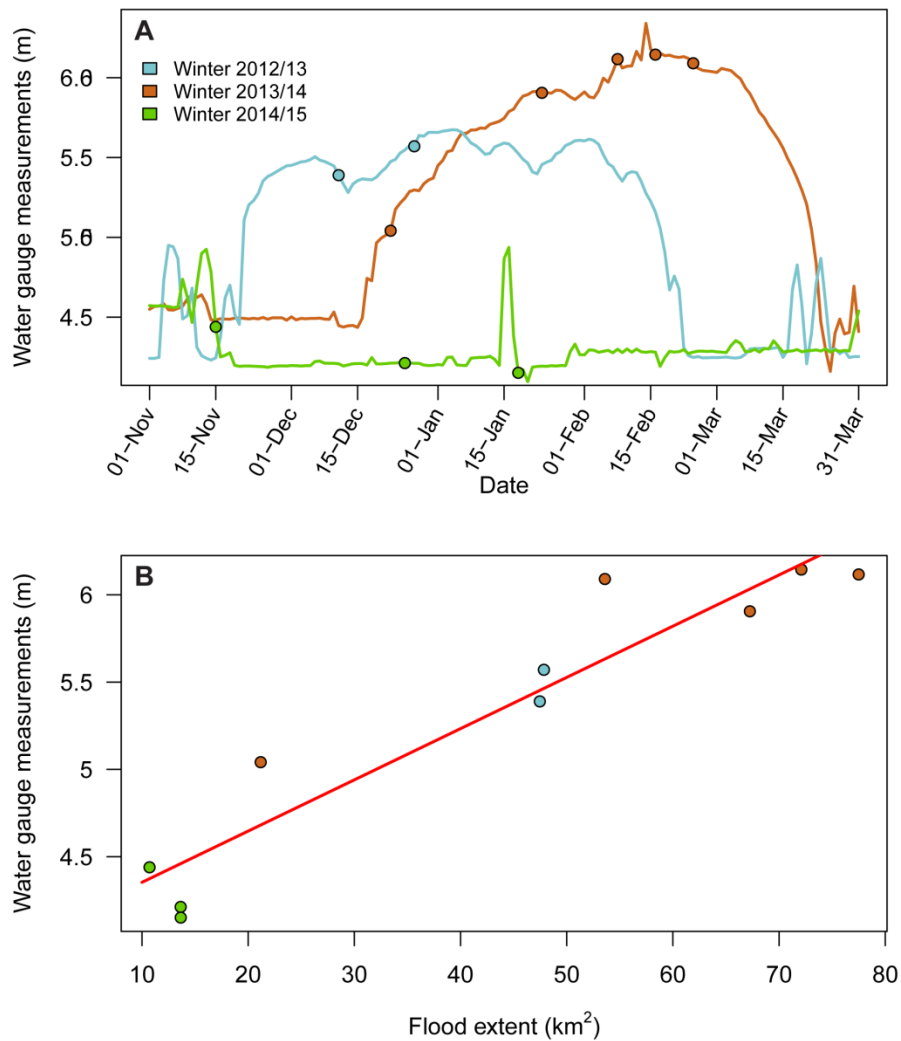


Fig 5.1: Panel (A): flood dynamics in the study site in the Somerset levels across the three winters. Points indicate dates with remote sensing data for the study area. Panel (B): relationship between water gauge measurements from the study area and the flood extent extracted from Landsat images, blue dots correspond to winter 2012–13, red dots to winter 2013–2014 and green dots to winter 2014–2015.

5.3.4 Bird distribution in response to flooding

Crane distribution in the study area was linked to flood extent, and during the peak of the extreme flood cranes restricted their foraging activity to a small area that was partially unflooded (Fig. 5.2D,E). We defined ‘preferred’ feeding areas as those that were used by cranes in winter 2014–15, when most of the area was unflooded and their foraging habitat was much less restricted. We used this

approach to account for seasonal variation in resource availability and to allow us to compare the same time points in each annual cycle. The distance between used areas and preferred areas was positively associated with flood extent (Table 5.1, Fig. 5.3A). The significant quadratic relationship between week of the season and distance from the preferred areas suggests that the difference in this distance among years decreased as the season progressed, but at a declining rate. In addition, there was evidence of temporal autocorrelation across birds in distance from preferred areas between weeks of the season within a given winter. However, when flood extent was accounted for, there was no difference in distance between winters and, based on model leave-one-out cross validation (LOO-CV) scores, winter was dropped from the best-fitting model. We found the same pattern for roost sites: the distance between used roost sites and preferred roost sites was positively associated with flood extent (Table 5.1, Fig. 5.3B). Cranes abandoned their usual roost areas and moved to partially flooded areas with shallow waters (Fig. 5.2). In contrast, distance between used and preferred roosting areas was not associated with week of the season and did not differ significantly between winters. Distance between used and the preferred roosting areas was autocorrelated across consecutive weeks of the season. In addition, there was less variation in this relationship during winter 2012–13 than in winter 2013–14.

5.3.5 *Bird energy expenditure and behaviour in response to flooding*

To investigate how daily energy expenditure changed in relation to flooding we used the overall dynamic body acceleration (ODBA hereafter) as a proxy (Gleiss, Wilson & Shepard 2011; Halsey, Shepard & Wilson 2011). We found a significant quadratic relationship between summed daily ODBA and flood extent in both winters (Fig. 5.4A,B). However, the nature of this relationship differed

significantly between winters, probably because of the different flood dynamics (Table 5.2). In winter 2012–13 the quadratic relationship was relatively flat but summed daily ODBA increased gradually with flood extension up to 30 km² before gradually decreasing (Fig. 5.4A). In contrast, during winter 2013–14, when the extreme flood occurred, summed daily ODBA generally increased with flood extent, however the rate of increase accelerated as flood extent rose above 60 km² (Fig. 5.4B). During the peak flood period ODBA was 15% higher than at low flood levels. Summed daily ODBA was also positively associated with Julian Date. In addition, there was evidence of temporal autocorrelation in daily ODBA across consecutive days. We also found evidence of significant among-individual and among-week variation in summed daily ODBA during the course of the study (Table 5.2).

The behaviour of birds was not significantly associated with flood extent during winter 2012–13, when water did not reach extreme levels (Table 5.3, Fig. 5.4C). In contrast, during winter 2013–14, when the extreme flood event occurred, there were significant quadratic relationships between flood extent and behaviour (Table 5.3). In particular, the probability of stationary behaviour decreased by 7% as flood extent increased whereas the probability of active behaviour increased by 8% at the peak of flood extent, which translates to around two extra hours of active behaviour each day (Fig. 5.4D). Stationary behaviour decreased as the winter progressed in both years. There was also significant among-individual variation in the probability of performing both stationary and flying behaviour in both years, suggesting that individual birds vary in the manner in which they respond to flooding events (Table 5.3).

Table 5.1: Generalized least square model of log-transformed distance between the used area and the preferred area during active and roosting periods. We defined ‘preferred’ feeding areas as those which were used by cranes in winter 2014–15, when most of the area was unflooded. Note that a p value cannot be calculated for the temporal autocorrelation structure (Corr. struct). We used leave-one-out cross validation (LOO-CV) scores to select the best model, including as predictors the weekly extent of flooding, the week in the winter, and winter. In the active period, based on model LOO-CV scores, the fixed effect was not included in our best fitting model but its coefficient is reported here for completeness. In the roosting period, the variance function allows within-group variance to differ between years. In this case the reported coefficient for the second winter represents the ratio between the standard deviation in the second winter relative to that in the first winter. Based on model LOO-CV scores week and winter were not included in the best model but coefficients are presented here for completeness. N = 44 observations.

Period	Variable	Coefficient	Lower 95% CI	Upper 95% CI	P-value
Active	Intercept	0.11	-1.26	1.47	0.87
	Flood Extent	0.99	0.42	1.56	< 0.001
	Week	-0.34	-0.67	-0.010	0.045
	Week ²	0.014	-0.0015	0.031	0.075
	Winter	-0.017	-1.88	1.85	0.95
	Corr. struct:	0.37	0.10	0.74	NA
	Week/Winter				
Roosting	Intercept	-0.18	-1.24	0.88	0.73
	Flood Extent	0.98	0.62	0.98	< 0.001
	Week	-0.035	-0.096	0.025	0.24
	Winter	0.32	-0.62	1.27	0.49
	Corr. struct:	0.31	-0.075	0.61	NA
	Week/Winter				
	Var. function: 2 nd	0.52	0.33	0.81	NA
	Winter				

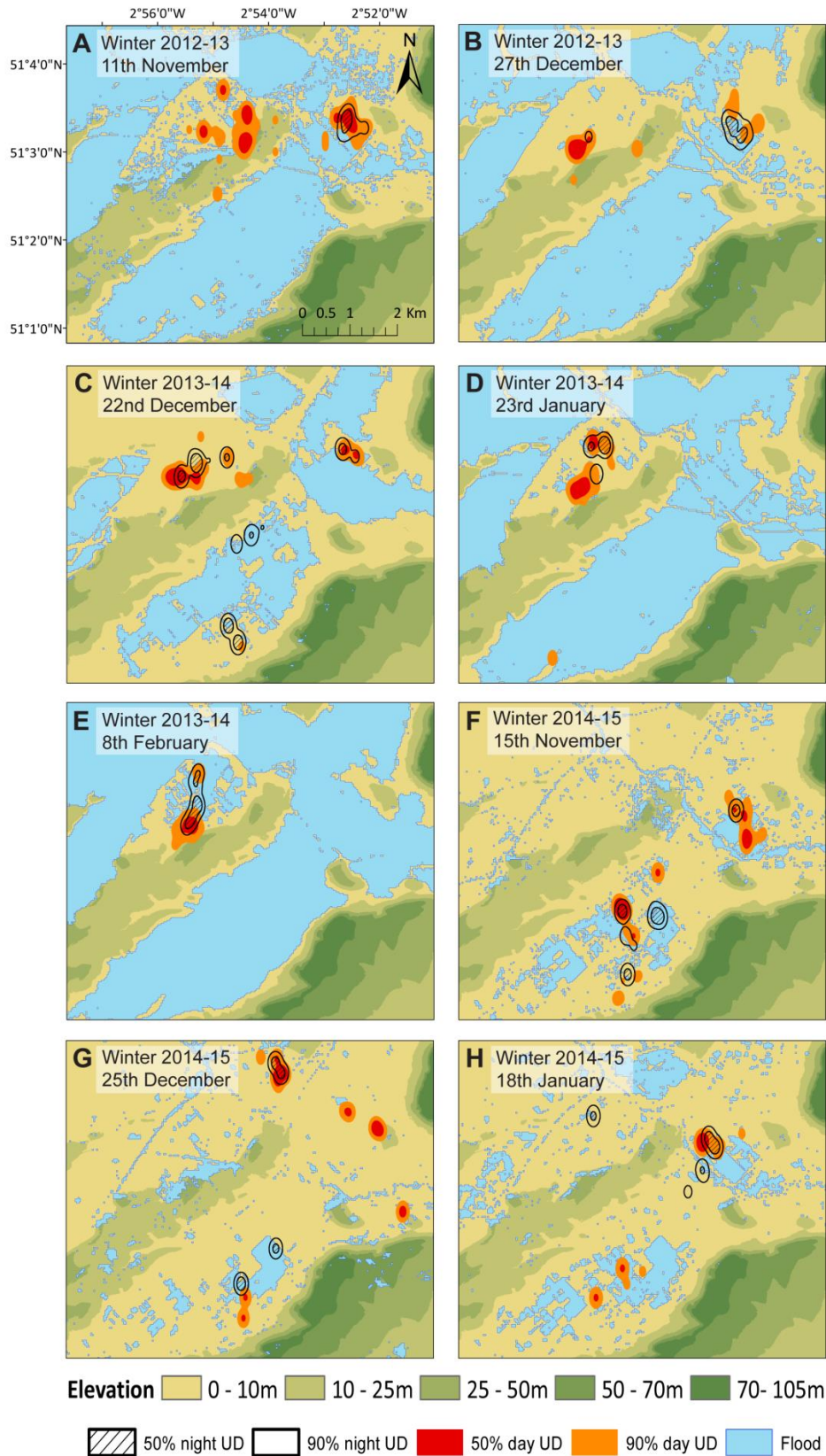


Fig 5.2: Crane active and roosting distribution as a function of flood extent for several dates across the three winters of study. Selected dates correspond to 9 out of 10 cloud-free satellite images that met the necessary requirements for measuring the extent of flooding. UD stands for utilization distribution. Map was created with ArcGIS version 10.2.2 (<https://www.arcgis.com/>).

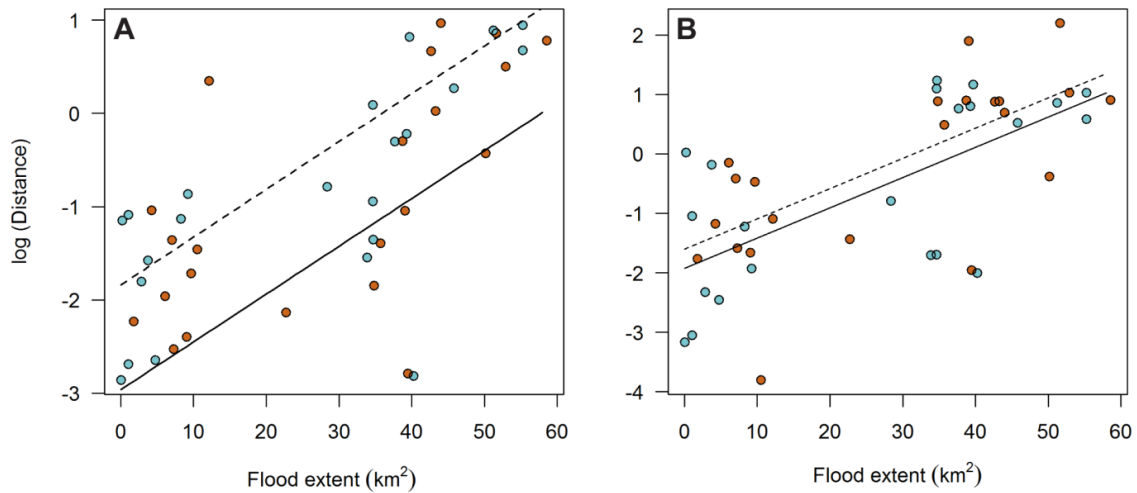


Fig 5.3: Fitted curves from the generalized least square model showing the relationship between flood extent and log-transformed distance between the used area and the preferred area for (A) active and (B) roosting periods by tracked cranes. We defined ‘preferred’ feeding areas as those which were used by cranes in winter 2014–15, when most of the area was unflooded. Black dots represent data from winter 2012–13, red dots represent data from winter 2013–14. Solid line represents the fitted curve in winter 2012–13 and the dashed line represents the fitted curve in winter 2013–14.

5.4 Discussion

Here we provide rare evidence of how closely monitored individual animals respond to an infrequent, catastrophic weather event of the type that appears to be increasing as our climate changes. Our study highlights the importance of combining population and individual responses to better understand how species respond to environmental fluctuations. We show that although the extreme weather event did not affect survival of birds, cranes abandoned favoured feeding and roosting areas, substantially increased feeding time and energy expenditure, and reduced resting time. Crane time- and energy-budget models suggest a non-linear response to flood extent, with the impact of floods only becoming apparent above a threshold that was exceeded in the extreme winter of 2013–14, but not in the more normal winters. This non-linear response

is likely explained by the relationship between flood extent and the extent of available (unflooded) foraging area. During the two normal winters, multiple unflooded areas were available even at peak flood whereas during the peak of the extreme flood event there was a dramatic reduction of the potential feeding areas available to cranes.

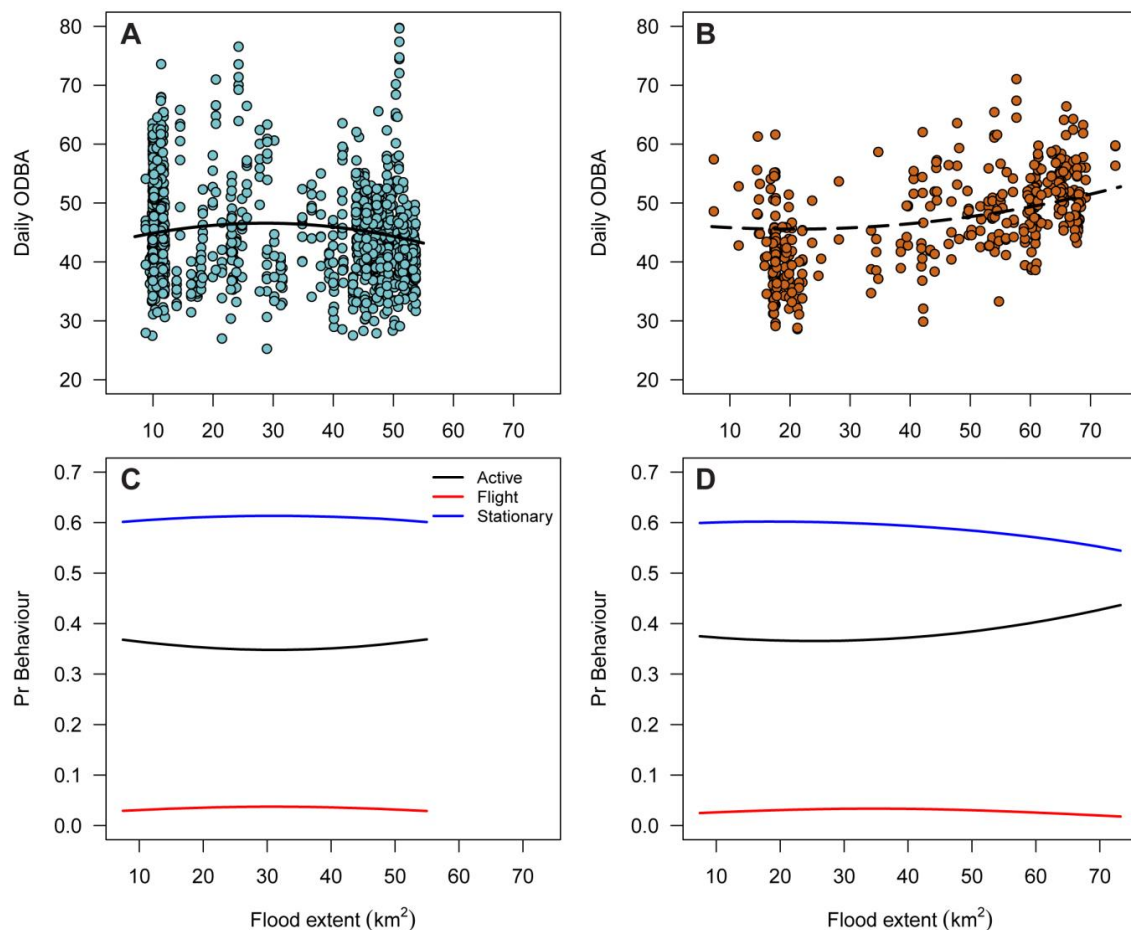


Fig 5.4: Upper panels: relationship between flood extent and individual daily energy expenditure (summed daily overall dynamic body acceleration), for normal winter 2012–13 (A) and extreme flood winter 2013–14 (B). Fitted curves from multinomial models predicted only over the range of observed data in each year. Lower panels: fitted curves from the multinomial model of behaviour in winter 2012–13 (C) and 2013–14 (D) showing the relationship between flood extent and the probability of performing one of three behaviours considered (active, flight or stationary).

Table 5.2: Linear mixed-effects model of daily ODBA. Random effects here represent among individual standard deviation in intercepts. Note that a p value cannot be calculated for the temporal autocorrelation structure (Corr. struct). Model selections was performed using K-fold cross validation where K = 5, including as predictors the daily extent of flooding, the Julian date in the winter, and winter. As random effects in the model we included bird ID and week. N = 1469 observations taken across two years. Number of weeks = 44. Number of birds = 11.

Variable	Coefficient	Lower 95% CI	Upper 95% CI	P- value
Intercept	46.02	43.63	48.41	< 0.001
Flood Extent	-1.27	-2.28	-0.27	0.0134
Flood Extent ²	-1.47	-2.61	-0.35	0.0104
Julian Date	3.59	3.062	4.13	< 0.001
Winter	-1.34	-6.041	3.42	0.53
Winter × Flood Extent	2.41	0.99	3.82	< 0.001
Winter × Flood Extent ²	2.48	0.91	4.061	0.002
Corr. struct: J. Date/Winter	0.057	0.018	0.18	NA
Var. function: 2 nd Winter	1.19	1.10	1.29	NA
Bird ID Random Effect	$\sigma = 2.89$	1.74	4.82	NA
Week ID Random Effect	$\sigma = 2.49$	2.011	3.091	NA

There are two non-exclusive potential reasons behind the increase in foraging time. First, during the extreme flood cranes were feeding in suboptimal areas with low food density, which might have forced them to spend progressively more time searching for food to meet their energy demands (Norberg 1997). Prolonged floods are known to have detrimental effects on soil macroinvertebrates, principally earthworms (a primary food of cranes), which can lead to a tenfold reduction in macroinvertebrate biomass in flooded areas compared with unflooded ones (Ausden, Sutherland & James 2001). Furthermore, flooded areas remain poor during the following spring due to

relatively slow re-colonization of soil invertebrates. Hence, our study population may have experienced a period of food shortage even after the main flood had receded and their preferred feeding areas were available (Ausden, Sutherland & James 2001). Second, the social organisation of flocking cranes may also provide an explanation for overall increases in foraging time during the extreme event. Dominant cranes tend to displace subordinate birds from higher quality areas (Bautista, Alonso & Alonso 1995). This in turn could generate increased searching in the displaced individuals and, indeed, we noted significant inter-individual differences in behaviours (Table 5.3).

Surprisingly, over-winter survival was extremely high during all three winters of study (~96%). The ability of individual cranes to adjust their time budgets (i.e., by increasing foraging effort and reducing resting time) seemed to be sufficient to buffer the effects of the flood. It is worth noting that during the extreme flood event temperatures were particularly mild, with average temperatures 2°C higher than the previous year for the whole period (Horfield and Filton weather station, Bristol, UK). This could have reduced thermoregulatory costs and thus allowed cranes to better cope with the flood. The lack of direct fitness consequences is consistent with another recent study that revealed that behavioural flexibility in black-tailed godwits (*Limosa limosa*) mitigated the potential carry-over effects of record low temperatures (Senner *et al.* 2015). Unfortunately, because of the small sample size we were not able to test the effects of floods on subsequent breeding success (cranes do not usually breed until 4 years of age and therefore most birds were reproductively immature both during the course of the extreme flood event and the subsequent breeding seasons). However, it has been documented that in heavily flooded areas, the sudden drop of water levels in spring can increase the risk of nest exposure to

predators in some ground-nesting meadow birds (Bellebaum & Bock 2009). Thus, the extreme flood could have had unexpected indirect effects on breeding success that we are unaware of.

Although short-term increases in energy expenditure had no apparent effects on immediate fitness in this instance, they have the potential to generate downstream consequences (Daan, Deerenberg & Dijkstra 1996). For example, increased energy expenditure during the breeding season has been associated with a twofold increase in mortality during the subsequent winter in kestrels (*Falco tinnunculus*, Daan, Deerenberg & Dijkstra 1996). Likewise, the heavier workload imposed on barn swallows (*Hirundo rustica*) by tracking devices and the correspondent increase in energy expenditure reduced annual survival, delayed reproduction in the subsequent year and diminished clutch size (Scandolara *et al.* 2014). In our case, the cumulative effect of short-term environmental stresses that will increase in frequency as a result of climate change are currently unclear, but they might influence the demography of populations facing such circumstances. This study highlights the complexity of linking population responses with a rapidly changing environment. The flood conditions experienced in the Somerset Levels during the winter 2013–14, were sufficiently short – and were likely partially alleviated by mild temperatures – to allow individuals to cope with them through behavioural flexibility. However, not all individuals were able to respond in the same way, indicating that there may be limits to this flexibility and it seems reasonable to assume that more extreme conditions could generate fitness consequences. At the population level, responses are likely to be variable and idiosyncratic, with some species being more vulnerable than others. Moreover, under different climate change scenarios, where many organisms are already challenged by other factors

(Root 2003; Pörtner & Farrell 2008), the capacity to respond to extreme events may already be compromised and it could lead to increased species vulnerability (Maron *et al.* 2015). At this stage it is difficult to generalise the results obtained here on the impact of extreme flooding given the paucity of studies that address the topic. Moreover, since the opportunities to monitor those events are mostly subject to chance, and because the responses tend to be non-linear, such studies are unlikely to become commonplace. However, we must take advantage of these unusual opportunities to build up our understanding of the consequences these events will have on ecosystems.

Table 5.3: Coefficients for Bayesian multinomial model of crane behavioural categories based on accelerometry data. Coefficients show the effect of predictors on the probability of performing stationary and flying behaviour respectively compared to active behaviour. Random effects represent among-individual standard deviation in the probability of performing flying and stationary behaviour respectively. Winter 2012–13: N = 126,840 observations from 7 birds. Winter 2013–14: N = 49,440 observations from 4 birds. Fixed effects where 95% CRI does not cross zero are highlighted in bold.

Winter	Variable	Coefficient	Lower 95% CRI	Upper 95% CRI
2012- 13	Flying	-2.48	-3.24	-1.74
	Stationary	0.62	-0.034	1.27
	Flying × Julian Date	0.033	-0.045	0.11
	Stationary × Julian Date	-0.12	-0.16	-0.077
	Flying × Flood Extent	-0.13	-0.26	0.010
	Stationary × Flood Extent	-0.031	-0.10	0.041
	Flying × Flood Extent ²	-0.20	-0.37	-0.031
	Stationary × Flood Extent ²	-0.051	-0.13	0.039
	Bird ID × Flying Random Effect	$\sigma = 0.86$	0.54	1.70
	Bird ID × Stationary Random Effect	$\sigma = 0.76$	0.47	1.48
2013- 14	Flying	-3.29	-4.36	-2.16
	Stationary	0.29	-0.73	1.29
	Flying × Julian Date	-0.036	-0.17	0.096
	Stationary × Julian Date	-0.18	-0.24	-0.13
	Flying × Flood Extent	-0.20	-0.34	-0.065
	Stationary × Flood Extent	-0.065	-0.12	-0.011
	Flying × Flood Extent ²	0.012	-0.14	0.18
	Stationary × Flood Extent ²	-0.053	-0.096	-0.011
	Bird ID × Flying Random Effect	$\sigma = 0.85$	0.47	2.22
	Bird ID × Stationary Random Effect	$\sigma = 0.81$	0.44	2.083

5.5 Methods

5.5.1 *Study site and fieldwork methods*

The Somerset Levels and Moors (51° 2' N, 2° 55' W; 56,650 ha) are situated in SW England. The landscape is primarily composed of wetlands and natural or semi-improved grasslands grazed by livestock in low densities, and secondarily of arable fields of cereals and maize. Between 2010 and 2014, 94 captive-reared juvenile Eurasian cranes were released in the area. Before release, all individuals were marked with individual colour-ring combinations, and several individuals from each cohort were fitted with telemetry tags. Overall we deployed 8 leg-mounted solar-powered GPS-PTTs (North Star ST LLC); the total attachment mass was 71.7 g in 2010 and 63.7 g in 2012. We also deployed 31 GPS-UHF tags (e-obs GmbH), which were backpack mounted using an elastic harness; the mass of the tag plus harness was 68 g. In 2013 and 2014 we fitted 4 birds with solar-powered GPS-GSM tags (Ecotone Telemetry), leg-mounted on standard crane colour-rings; the overall mass was 51.5 g. The mass of the heavier devices (GPS-PTTs in 2010) only represented 1.3% of average cranes body mass (5400 g). To ensure that individuals could acclimate to the transmitter, the attachment was performed several days before the release and no side effects were observed after release in the study birds. Duty cycles differed among transmitters: PTT and GSM tags transmitted locations every 1–3h, whereas GPS-UHF tags collected locations every 7 h. All location fixes from PTT tags were retrieved via the CLS tracking system (www.argos-system.org) and only standard class locations (3, 5) were retained. GPS-UHF tags recorded tri-axial body acceleration every 4 minutes in tags deployed in 2012 and 2013 and every 5 minutes in tags deployed in 2014. We

did not include in the analysis the acceleration data from tags deployed in 2014, because, as explained in the results section, these tags had different characteristics and their sensitivity was higher so the acceleration patterns were not consistent with the previous tags. Bird handling and tagging protocols were carried out in accordance with relevant guidelines and regulations. These protocols were approved by the Wildfowl & Wetlands Trust Animal Welfare and Ethics Committee and the British Trust of Ornithology.

5.5.2 *Survival and reproduction*

To estimate crane survival we estimated the proportion of birds that survived over winter (i.e known to be alive at start of the winter that were known to be alive at end of the winter). We searched and identified individually colour-ringed birds on a monthly basis for five consecutive months each winter, from the beginning of November to the end of March. This population is very localised, so the monthly surveys that have been carried out since the beginning of the reintroduction project in 2010, have high monthly resighting probabilities (ca. 94% monthly resighting probability), which obviates the need to control for resighting heterogeneity. Over the whole period no bird that has been unaccounted for more than five months has ever been resighted subsequently, therefore we considered a bird to be dead either when it was not recorded for more than 6 months or when its body was found. To test if there were significant differences in the survival rates among the three winters we used Test of Equal Proportions. We chose this approach because of the relatively invariant survival rates over the short duration of the study. Given the small sample size and the low number of individuals that died every winter it was not possible to include the age structure of the population in the analysis. We also performed surveys

during three breeding season, from 2013 to 2015, to assess the effect of winter conditions on subsequent breeding season. However, cranes begin to establish pairs in their second or third year, with successful breeding occurring in the fourth or fifth year (Archibald & Meine 1996). Thus, in 2013, the oldest birds in the population were only 3 years old and did not breed. This factor, combined with the small sample size from the following two breeding seasons, prevented us from performing statistical analysis to establish if floods had had an effect on breeding success.

5.5.3 *Flooding estimates*

We generated flood maps from multi-temporal Landsat 7 and Landsat 8 Operational Land Imager (OLI) images between the winters of 2012–13 and 2014–15. The main limitation we faced was the low availability of cloud-free images and the number of images affected by the failure of the Scan Line Corrector (Jung *et al.* 2011). Before any calculations were carried out, we converted Landsat 7 digital numbers (DN) into top of atmosphere (TOA) reflectance (NASA 2009). Similarly, Landsat 8 digital numbers were converted into TOA reflectance (USGS 2015). We used the Modified Normalized Difference Water Index (MNDWI), which uses one green band and one SWIR band to estimate the flooded surface (Xu 2006). The resulting maps were fed into the ISODATA algorithm in order to obtain a more detailed division of spectral classes. Subsequently, the classes corresponding with flooded surface were identified and selected. All GIS operations were carried out in QGIS 2.0 and ArcGIS 10.1.

To estimate the flood progression, we collected concurrent data from the water gauges in the study area. We used a generalized linear model to correlate the

water gauge measurements with the flood extents extracted from the flood maps. Then we inferred flood extent from gauge measurements and obtained a dynamic model of flood progression over the three winters.

5.5.4 *Bird distribution in response to flooding*

To characterize the daily (foraging) and nightly (roosting) distribution of cranes during the three consecutive winters, we used utilization distribution (UD) kernels (smoothing 0.001 and grid 2000). We estimated all kernels using *adehabitat* R package (Calenge 2006). To assign each location to either active or roosting period we used the accelerometry data. We found a correlation between time of sunrise and the start of the active period ($p < 0.0001$) and between time of sunset and the start of the roosting period ($p < 0.0001$), so we were able to establish a threshold between the active period and the roosting period. The active period started ca. 63 minutes before sunrise and the roosting period started ca. 83 minutes after sunset.

To assess the impact of the flood extent on crane displacement from their preferred areas, we separately calculated the weekly 50% UD kernels for the active and the roosting period for the three winters. We then calculated the centroids of these kernels. Since the winter 2014–15 was particularly dry we used it as a reference to determine the preferred areas because the flood extent was relatively low and birds were free to use almost the whole area. We then calculated the minimum distance between the centroids for the winters 2012–13 and 2013–14 and the centroids of the preferred areas. We decided to use the minimum distance between the used areas and the preferred areas in order to focus on the displacement from the core areas and avoid the effect of exploratory behaviour far from the usual areas. To account for the possible

variation in habitat among years we performed a complementary analysis, setting the sites used during the first week of November of winter 2012–13 and 2013–14 (instead of the whole winter 2014–15) as reference for preferred areas. We then calculated the minimum distance between those areas and the areas used in the subsequent weeks for the two winters. Analyses for both models were performed in the same manner. Results are qualitatively similar to those obtained in the previous model, thus only the first model is explained in the results section (Supplementary Table 5.S1).

To examine whether the extent of flooding throughout the winter was linked to the distance between preferred sites (for active periods) we fitted a generalized least squares (GLS) model with log-transformed distance as our response variable in the R environment (R. Core Team 2014) using the package *nlme* (Pinheiro *et al.* 2014). As fixed effect predictors in our model we included the weekly extent of flooding, the week in the winter, and winter (as a categorical variable). Prior to analysis, flood extent was standardized, to set the intercept to intermediate flood levels and ease model interpretation. Because both flood extent and week may have non-linear effects, we ran different models in which these continuous terms were included as simple linear effects, or as non-linear by fitting either a quadratic fixed effect or using b-splines. In addition, we included in our full model all two-way interactions between our fixed effects. Finally, we included an autocorrelation structure of order 1, using week as a continuous time covariate with year as the relevant grouping factor. This accounts for temporal autocorrelation between observations from adjacent weeks within a year. Model selection was performed using leave-one-out cross validation (LOO-CV) and selecting models with the lowest mean squared error

(MSE) and predicted residual sum of squares (PRESS) scores (Picard & Cook 1984).

To model whether the extent of flooding had an effect on the distance between preferred roosting sites and used roosting sites, we used the same GLS modelling approach as described above, with log distance between preferred and used roosting sites as our response variable. However, due to evidence of heteroscedasticity in model residuals we also incorporated a variance function to allow for heterogeneity in residuals across the three years of our study (Cleasby & Nakagawa 2011).

5.5.5 *Bird energy expenditure and behaviour in response to flooding*

To investigate how daily energy expenditure changed in relation to flooding we used overall dynamic body acceleration (ODBA) as a proxy and calculated the summed ODBA of every tagged bird on a daily basis (Gleiss, Wilson & Shepard 2011; Halsey, Shepard & Wilson 2011). We used this measure as a response variable in a linear mixed effects model (LMM). As fixed effects in our model we included the daily flooding extent, the day of the year (measured as Julian date from the start of the appropriate year) and the winter in which records were taken. The continuous variables, daily extent of flooding and Julian date, were incorporated into models as linear effects or as non-linear effects by fitting either a quadratic curve or using b-splines. All two-way interactions between variables were also included in our full models before simplification took place. We included bird ID as a random effect to account for the fact that summed daily ODBA measures taken from the same individual may not be independent, and week of winter to account for the lack of independence of measures taken within the same week. In addition, we included a temporal autocorrelation

structure of order 1 using Julian date as a continuous time covariate, and with year as the relevant grouping factor, to account for temporal autocorrelation in summed daily ODBA values. Due to evidence of heteroscedasticity in model residuals we also allowed the residual variance in our models to differ across years. Model selection was performed using K-fold cross validation where $K = 5$ in order to calculate MSE and identify the best fitting model. K-fold cross validation represents a variation on LOO-CV and was developed as an alternative to the computationally expensive LOO-CV (Geisser 1975) because of the large number of data points in the ODBA models.

To elucidate bird behaviour through acceleration patterns, we used AcceleRater, a web application that provides supervised machine learning models that classify unknown behaviour through already labelled data (Resheff *et al.* 2014). We classified crane behaviour into three categories: stationary, active and flying. To do so, we filmed birds fitted with GPS-UHF tags. We obtained 118 instances of stationary behaviour, 310 of active behaviour and 89 of flying behaviour. We ran several different fitting techniques (K nearest neighbours, Linear support vector machines, Radial basis function kernel support vector machines, Decision tree, Random forest and Naïve Bayes) using these data. The results were cross validated with the Train-Test split method to assess the recall, accuracy and precision of the output of a given model. We selected the Random Forest method to classify the unlabelled data since it obtained the highest scores in recall, precision and accuracy, 95.8%, 96.0% and 97.4% respectively. Each crane behaviour-type was classified in this way every 15 minutes, generating a vector of behavioural responses Y_i that take one of $J = 3$ discrete values. With such categorical data the multinomial distribution

can be used to estimate the probability that the i th response falls into the j th category (equation 1).

$$\pi_{ij} = \Pr\{Y_i = j\} \quad (1)$$

Where, for example, π_{i1} is the probability that i th response is classed as 'active'. Moreover, because our response categories are mutually exclusive and exhaustive we have:

$$\sum_{j=1}^J \pi_{ij} = 1 \quad (2)$$

That is, the probabilities sum to one for each response and we have $J-1$ parameters because once we know the probability of Y_i being classed as active and of being classed as flying then by simple subtraction we know the probability that Y_i will be classed as stationary (equation 2). To model the behaviour of cranes we used a Bayesian multinomial mixed-effects model within the *MCMCglmm* R package (Hadfield 2010), with three response variables, active, flying and stationary. As fixed effects within our model we included Julian date, winter and daily flood extent. Visual inspection of data suggested the inclusion of a quadratic flood extent effect and an interaction between flood extent and winter. Initially, we incorporated a two-way interaction between flood extent and winter in our multinomial models. However, once the existence of a two-way interaction between winter and flood extent was confirmed (95% CRI of interaction coefficient did not overlap 0) we ran separate multinomial models for each winter of our dataset for ease of subsequent interpretation. By doing so, we could still capture the interaction as we were still modelling separate flood extent coefficients for each year. As random effects in our models we included bird ID and Julian date to account for the potential lack of independence on measurements taken on the same bird or the same day. We used non-

informative priors in our multinomial models and ran 3 MCMC chains for 150,000 iterations, with a burn-in of 30,000 and a thinning interval of 10 (Hadfield 2010). Convergence of chains was assessed using the Gelman-Rubin diagnostic and model fit was assessed using a posterior predictive check (Gelman & Rubin 1992).

As crane behaviour was assessed (via accelerometry) every 15 minutes, consecutive behaviours in time were likely to be highly correlated; we thus added a lagged dependent variable (the behaviour previously performed by the bird) to the original model to reduce the occurrence of autocorrelation. Results are similar to those obtained in the original model and our conclusions are thus unaffected by potential behavioural autocorrelation (Supplementary Table 5.S2).

5.6 Supporting Information

Supplementary Table 5.S1: Generalized least square model of log-transformed distance between used areas: (1) during the first week of November of 2012 and the remaining weeks of the winter 2012-2013 and (2) during the first week of November of 2013 and the remaining weeks of the winter 2013-2014, for the active and roosting periods. We chose the first week of November of each year as a reference for preferred area because most of the area was unflooded and birds were free to use the whole study area. Note that a p value cannot be calculated for the temporal autocorrelation structure (Corr. struct). We used leave one out cross validation (LOO-CV) scores to select the best model, including as predictors the weekly extent of flooding, the week in the winter, and winter. In the roosting period, the variance function allows within-group variance to differ between years. In this case the reported coefficient for the second winter represents the ratio between the standard deviation in the second winter relative to that in first winter. Based on model LOO-CV scores winter was not included in the best model but the coefficient is presented here for completeness. N = 42 observations.

Period	Variable	Coefficient	Lower 95% CI	Upper 95% CI	P-value
Active	Intercept	-2.55	-3.43	-1.66	< 0.001
	Flood Extent	0.029	0.011	0.046	0.0018
	Flood Extent ²	-0.001	-0.003	-0.0001	0.032
	Week	0.13	0.068	0.18	< 0.001
	Winter	0.76	-0.002	1.53	0.051
	Corr. struct: Week/Winter	0.36	0.091	0.76	NA
Roosting	Intercept	-2.28	-3.78	-0.77	0.004
	Flood Extent	0.041	0.068	0.10	< 0.001
	Week	0.058	-0.008	0.18	0.07
	Winter	0.83	-0.49	2.16	0.21
	Corr. struct: Week/Winter	0.51	0.21	0.80	NA
	Var. function: 2 nd Winter	0.68	0.44	1.06	NA

Supplementary Table 5.S2: Coefficients for Bayesian multinomial model of crane behavioural categories based on accelerometry data. Coefficients show the effect of predictors on the probability of performing Stationary and Flying behaviour respectively. Random effects represent among-individual standard deviation in the probability of performing Flying and Stationary behaviour respectively. To account for the existence of autocorrelation in the model we included lagged dependent variables ($t-1$). Winter 2012-13: N = 126,833 observations from 7 Birds. Winter 2013-14: N = 49,436 observations from 4 Birds. Fixed effects where 95% CRI does not cross zero highlighted in bold.

	Variable	Coefficient	Lower 95% CRI	Upper 95% CRI
2012-13	Flying	-2.73	-3.44	-2.03
	Stationary	-0.97	-1.47	-0.41
	Flying \times Julian Date	0.041	-0.033	0.11
	Stationary \times Julian Date	-0.071	-0.11	-0.042
	Flying \times Flood Extent	-0.12	-0.25	0.010
	Stationary \times Flood Extent	-0.022	-0.083	0.003
	Flying \times Flood Extent ²	-0.19	-0.35	-0.036
	Stationary \times Flood Extent ²	-0.037	-0.11	0.039
	Flying \times Flying $_{t-1}$	0.74	0.62	0.86
	Stationary \times Flying $_{t-1}$	0.18	0.091	0.26
	Flying \times Stationary $_{t-1}$	0.50	0.43	0.58
	Stationary \times Stationary $_{t-1}$	2.81	2.77	2.84
	Random Effects			
	Bird ID \times Flying	$\sigma = 0.83$	0.53	1.62
	Bird ID \times Stationary	$\sigma = 0.66$	0.41	1.26
2013-14	Flying	-3.45	-4.51	-2.37
	Stationary	-1.31	-2.30	-0.28
	Flying \times Julian Date	-0.0021	-0.15	0.11
	Stationary \times Julian Date	-0.11	-0.16	-0.071
	Flying \times Flood Extent	-0.19	-0.33	-0.065
	Stationary \times Flood Extent	-0.057	-0.10	-0.005
	Flying \times Flood Extent ²	0.051	-0.15	0.17
	Stationary \times Flood Extent ²	-0.043	-0.078	-0.006
	Flying \times Flying $_{t-1}$	0.72	0.44	0.99
	Stationary \times Flying $_{t-1}$	0.26	0.10	0.43
	Flying \times Stationary $_{t-1}$	0.51	0.37	0.65
	Stationary \times Stationary $_{t-1}$	3.02	2.97	3.07
	Random Effects			
	Bird ID \times Flying	$\sigma = 0.83$	0.45	2.18
	Bird ID \times Stationary	$\sigma = 0.79$	0.43	2.08

Chapter 6

General discussion

6.1 Overview

Understanding the processes that shape population size and structure is a key area in ecology and conservation (Begon, Harper & Townsend 1996; Levin *et al.* 2009). Any conservation strategy that aims to increase the survival prospects of a population must be predicated upon accurate knowledge of the population dynamics in order to be successful (Levin *et al.* 2009). Thus, disentangling the combined effect of survival, productivity and dispersal should be at the core of any conservation plan. For small and recently established populations, assessing such demographic traits is particularly relevant since stochasticity plays a much more important role in the relative contribution of survival and productivity to population growth and can rapidly drive the population to extinction (Lande, Engen & Saether 2003).

A primary aim of this thesis, therefore, was to understand the population dynamics of Eurasian cranes in the UK and predict its future population size and distribution. To do so I tried to disentangle the confounding effects of survival, productivity and dispersal in population growth rate. I analysed dispersal through two approaches: a demographic approach, where I tried to determine the relative contribution of birds from continental sources; and a spatial one, where I tried to establish the dispersal prospects of the population within England and the environmental factors affecting them.

Additionally, in this thesis, I have covered other aspects of crane conservation in the UK, which were of considerable interest to the CASE (Collaborative Award in Science and Engineering) partner (Wildfowl & Wetlands Trust). First, I tried to assess the levels of public engagement with the first successful breeding attempt of cranes in the SW England after 400 years through Internet-

based methods. In addition, I assessed the resilience of cranes to extreme weather events to understand how they might cope with environmental challenges, specifically flooding.

The ecology of cranes is particularly relevant to study in the UK because of its historic circumstances and its status as a flagship species. The extirpation of the population in the 16th century and its subsequent slow recovery offered a unique opportunity to study population dynamics, colonization rates and public response to conservation efforts. In addition, the reintroduction of 90 individuals in the Somerset Levels to enhance the population recovery provided an excellent framework to assess the effect of population reinforcements on colonization and population dynamics.

6.2 Summary of key findings

In **chapter 2**, I aimed to assess the relevance of Internet-based methods in capturing conservation awareness and as such I focused on: a conservation outcome of the crane reintroduction project (the first successful breeding attempt in SW England in over 400 years); two of the conservation NGOs involved in the reintroduction project (the Royal Society for the Protection of Birds (RSPB) and the Wildfowl & Wetlands Trust (WWT)); and the reintroduction of the red kite (*Milvus milvus*) across the UK. I found that Internet-based methods are useful to track changes in public interest in conservation across time and space. Particularly, onsite metrics, which monitor visitors' traffic within websites, offer a high degree of accuracy and flexibility. Such methods have a range of applications for conservation biologists and managers, from monitoring project success in engaging people in conservation, to

understanding intricate drivers of behaviour of people in relation to conservation awareness.

In **chapter 3**, I assessed the population dynamics of cranes in the UK and determined the potential effect of reinforcement on population growth. I found that the crane population appears to be acting as a pseudo-sink, with immigration playing a major role in population recovery. The current levels of survival and productivity support the population's persistence; but, without immigration the rate of increase would be substantially lower. Furthermore, measures to improve the productivity rates, such as adequate management of water levels, should be implemented, since the productivity of the UK population (0.56) is lower than the productivity of other populations in continental Europe (0.9) (Mewes, Prange & Nowald 2010). The reintroduction has increased the projected population size by 50% over the next 50 years and has decreased the importance of immigration; by 2065, there could be around 275 breeding pairs in the UK if the modelled survival, immigration and productivity rates are maintained.

Evidence suggests that the population is going to increase in the coming years. Thus, in **chapter 4**, I explored how this increase in population size could translate into an expansion of the population range in England. Using log-Gaussian Cox process models, I showed that cranes prefer large wetlands with reduced perimeter-to-area ratios. However, the proximity to already colonized areas is more important in determining the potential colonization areas than the spatial characteristics of the wetlands, i.e. cranes are more likely to colonize wetlands adjacent to established breeding areas irrespective of their environmental characteristics. Consequently, the reintroduction of cranes in SW

England could be viewed as a human-assisted long-distance dispersal event that has allowed the population to spread westward and has increased the geographical range of the potential wetlands to be colonized.

In this context, where cranes are still in the first stages of population recovery and population numbers remain low, it is particularly relevant to explore the resilience of cranes to environmental perturbations. In **chapter 5** I explored the response of cranes to extreme weather events, to do so I monitored survival, distribution, energy expenditure and behaviour of the reintroduced cranes during three consecutive winters (2012–2015); the second of which had the most extensive floods in over 200 years in SW England. I found that during the peak of the 2013–14 floods crane foraging areas became limited to a small partially unflooded patch. They also increased energy expenditure (+15%) through a reduction in resting time and an increase in foraging time. Survival did not differ among the three winters, which indicates cranes withstood the extreme weather event through behavioural plasticity, modifying their time-energy budgets.

In summary, evidence suggests that the crane population in the UK is going to continue its recovery in the coming years; however, it is likely that the new individuals will remain close to the already colonized areas. Nevertheless, conservation measures to increase the population productivity and habitat suitability should be implemented to enhance the complete recovery of the population.

6.3 Broader social and ecological implications

The impact of humans on the world's ecosystem has led to a continuous decline of global biodiversity; one quarter of all vertebrate species could become extinct within the next century (Baillie *et al.* 2010). Conservation successes and species recovery are rare compared to the general failure to meet the conservation targets set to reduce biodiversity loss (Butchart *et al.* 2010). Successful conservation is essential to ensure the sustenance of ecosystems processes that provide key services to society (Rands *et al.* 2010).

Understanding the underlying mechanisms allowing population recovery can offer solutions to reverse human-induced declines in other populations. High adult survival in the crane population in the UK combined with positive net immigration from continental Europe has led to the population recovery in the UK. Thus, conservation measures implemented in continental Europe to protect cranes are likely to have driven the population recovery in the UK, which highlights the complexity of planning conservation strategies and the importance of international cooperation to tackle the biodiversity crisis.

In addition, the recovery of particular species plays an important role in terms of conservation engagement, since they provide evidence that conservation action can achieve positive results and can promote pro-environmental behaviour among the human population (Ojala 2012). A constructive attitude towards conservation challenges is imperative, since hopelessness and pessimism may lead to inactivity and denial (Ojala 2012). Particularly the recovery of emblematic species, such as the Eurasian crane, is important to enhance the participation of local communities in conservation (Borrini & Dianne 1997; Bowen-Jones & Entwistle 2002). We showed that there is a geographic gradient in interest in the crane and the red kite reintroduction projects; people from

areas near to the projects have a greater interest than people from areas further away. This information is extremely relevant to assess the degree of involvement among local communities and design appropriate conservation awareness campaigns.

It is also clear that the study of individual-level responses is essential to assess population vulnerabilities. To do so, long-term individual based studies are necessary since they provide the possibility to explore a wide range of ecological responses and a reliable basis upon which to assess the effect of changes in environmental parameters upon individuals and in turn the population (Clutton-Brock & Sheldon 2010). The crane population in the Somerset Levels has been intensely monitored since the first cohort of birds was released in 2010. In addition, all birds have been equipped with either radio-tags or telemetry devices. This allowed me to take advantage of a unique opportunity to study individual responses to the most extraordinary flood event in the Somerset Levels since the 19th Century, and revealed the coping mechanisms of cranes and their resilience in face of extreme weather challenges.

The recent technological and scientific advances in species monitoring is generating a massive body of information about individual distribution and characteristics; however, transforming this data into useful conservation evidence is still challenging. I provide an additional alternative to model species distribution: spatio-temporal log-Gaussian Cox process models. This approach is particularly useful for dynamic populations when information about life history traits is missing. Nevertheless, these models are in the early stages of

development, thus their range of capabilities is likely to increase in the near future.

In addition, I hope that new ground-breaking methods will facilitate and diversify the information available on small populations. For example, molecular techniques, such as the analyses of environmental DNA from nest remains, could help to answer a range of ecological questions. It would enable the creation of a population pedigree, which in turn would support the assessment of the population origin, the level of inbreeding and the actual rate of immigration. Moreover, in case of breeding failure due to predation it would allow us to identify the predator and establish appropriate management strategies.

In this thesis I have tried to answer a wide range of ecological questions related to the crane recovery and reintroduction in the UK and derive broader implications for animal conservation. Nevertheless, several questions about crane population dynamics in the UK remain unanswered, mainly due to the small size of the population and the resultant small sample sizes. Therefore, in terms of the species conservation, a priority for future work (when enough information can be gathered) is to understand the causes of the low productivity rates in the population and develop management strategies to reduce predator impacts on nesting birds. Additionally, detailed information from continental crane populations could be useful to develop metapopulation models to understand how changes in continental populations could shape the crane population in the UK.

Appendix: Additional publications

Gutiérrez, J. S., Soriano-Redondo, A., Dekinga, A., Villegas, A., Masero, J. A. & Piersma, T. (2015) How salinity and temperature combine to affect physiological state and performance in Red Knots with contrasting non-breeding environments. *Oecologia*, **178**, 1077-1091.

Soriano-Redondo, A., Cortés, V., Reyes-González, J.M., Guallar, S., Bécares, J., Rodríguez, B., Arcos, J.M. & González-Solís, J. (2016) Relative abundance and distribution of fisheries influence risk of seabird bycatch. *Scientific Reports*, **6**, 37373.

How salinity and temperature combine to affect physiological state and performance in red knots with contrasting non-breeding environments

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Abstract Migratory shorebirds inhabit environments that may yield contrasting salinity-temperature regimes—with widely varying osmoregulatory demands, even within a given species—and the question is: by which physiological means and at which organisational level do they show adjustments with respect to these demands? Red knots *Calidris canutus* winter in coastal areas over a range of latitudes. The nominal subspecies winters in salty areas in the tropics, whereas the subspecies *Calidris canutus islandica* winters in north-temperate regions of comparatively lower salinities and temperatures. In this study, both subspecies of red knot were acclimated to different salinity (28/40 ‰)—temperature (5/35 °C) combinations for 2-week periods. We then measured food/salt intakes, basal metabolic rate (BMR), body mass and temperature, fat and salt gland scores, gizzard mass, heat-shock proteins, heterophils/lymphocytes (H/L) ratio and plasma Na⁺ to assess

the responses of each taxon to osmoregulatory challenges. High salinity (HS)-warm-acclimated birds reduced food/salt intake, BMR, body mass, fat score and gizzard mass, showing that salt/heat loads constrained energy acquisition rates. Higher salt gland scores in saltier treatments indicated that its size was adjusted to higher osmoregulatory demands. Elevated plasma Na⁺ and H/L ratio in high-salinity-warm-acclimated birds indicated that salt/heat loads might have a direct effect on the water-salt balance and stress responses of red knots. Subspecies had little or no effect on most measured parameters, suggesting that most adjustments reflect phenotypic flexibility rather than subspecific adaptations. Our results demonstrate how salinity and temperature affect various phenotypic traits in a migrant shorebird, highlighting the importance of considering these factors jointly when evaluating the environmental tolerances of air-breathing marine taxa.

Keywords Osmoregulation · Phenotypic flexibility · Shorebird · Thermoregulation · Water–salt balance

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Introduction

Osmotic homeostasis, i.e. the maintenance of a relatively constant salt and water balance in the body fluids, is one of the best-defended physiological states in vertebrates (Evans 2009; Bradley 2009). Amphibians, reptiles, birds, and mammals typically have blood concentrations of around 250–300 mOsm to ensure proper functioning of cells (Bradley 2009). The problem of maintaining the internal environment within fairly well-defined limits varies with the degree of gain or loss of salts and water as a result of environmental pressures (Phillips and Ensor 1972). Therefore, the ability to identify conditions when osmotic

homeostasis is not achieved, or is compromised in the face of conflicting ecological demands, could be a means to identify periods of environmental stress (Gutiérrez 2014).

In marine and other saline environments, organisms may face problems of water conservation because of high salt concentration. Moreover, these problems could be compounded by factors such as temperature, which additionally demands physiological and behavioural adjustments to maintain homeostasis in terms of salt and water balance (e.g. Phillips and Ensor 1972; Skadhauge 1981; Battley et al. 2003; Gutiérrez et al. 2012). In nature, salinity and temperature often vary in concert. In considering the potential impacts of climate-induced changes in these (and other) abiotic factors, it is important to understand their influence on the physiological performance of organisms and, ultimately, on fitness (e.g. Pörtner 2002; Bozinovic et al. 2011). Nevertheless, the combined effects of these abiotic factors have been largely overlooked in aquatic air-breathing vertebrates. By using large-scale biogeographical analyses and environmental tolerances, Brischoux et al. (2012, 2013a) showed that salinity and temperature influence the distribution and dispersal of marine snakes (*Laticauda* spp.), with the species with the more efficient salt glands and lower dehydration rates exploiting more saline oceanic areas.

This may also apply to shorebirds (Charadriiformes, suborders Charadrii and Scolopaci) undertaking seasonal migrations between the far north and further south (e.g. van de Kam et al. 2004), during which they are subject to large changes in the salinity and temperature of their experienced environments. Outside the breeding season, the species that opt to winter in coastal habitats (Piersma 1997) feed on marine invertebrates [typically isosmotic to surrounding seawater (Prusch 1983)] and have limited or no access to freshwater (Gutiérrez 2014). To cope with salt loads, shorebirds—like marine birds—use renal and extrarenal mechanisms which jointly ensure the excretion of excess salt without a net loss of water (Peaker and Linzell 1975; Goldstein and Skadhauge 2000; Gutiérrez 2014). The phenotypic flexibility in osmoregulatory structures (e.g. supraorbital salt glands) is critically important in allowing migratory shorebirds to successfully overcome the osmoregulatory challenges faced in the course of their annual cycles (Gutiérrez et al. 2012). Nevertheless, little is known about the physiological and ecological consequences of living in saline environments and the situations in which osmotic homeostasis is compromised (Gutiérrez 2014).

For example, it has been shown that two shorebird species (red knots *Calidris canutus* and sanderlings *Calidris alba*) wintering at Banc d'Arguin in Mauritania, a coastal wetland on the boundary between the Sahara and the Atlantic Ocean, reduced their food intake when switched from

fresh to seawater under experimental conditions, suggesting that salinity may constrain food intake and thus energy acquisition (Klaassen and Ens 1990). Indeed, recent work on red knots has shown that individuals maintained on seawater have lower food and water consumption rates than those having freshwater available (Oudman et al. 2014). In addition, there is evidence that an increase in salinity substantially increases the basal metabolic rate (BMR) of dunlins *Calidris alpina*, which demonstrates that osmoregulation entails significant energetic costs (Gutiérrez et al. 2011; see also Nelms 1996 and Peña-Villalobos et al. 2013). Other studies have also shown that shorebirds can suffer adverse effects when living in saline environments (e.g. Purdue and Haines 1977; Hannam et al. 2003; Gutiérrez et al. 2013). However, salinity is not the only factor challenging osmotic homeostasis. By affecting physiological routes of heat/water loss, temperature also exerts a considerable influence on the osmoregulatory performance of birds (e.g. Phillips and Ensor 1972; Skadhauge 1981; Verboven and Piersma 1995; Gutiérrez et al. 2012). At similar salinities, the salt gland mass of red knots increases at both low and high temperatures, which probably reflects increased energy demands at low temperatures (i.e. higher food/salt intake) and elevated water loss at high temperatures (Gutiérrez et al. 2012). Taken together, these results reveal that changes in both salinity and temperature can have significant effects on shorebirds' physiological state and performance.

Long-distance migrant shorebirds breeding in the high Arctic and wintering along tropical coasts may encounter a greater range of conditions (e.g. in terms of salinity and temperature) compared with temperate-wintering birds. An interesting contrast in this respect is provided by two subspecies of red knots: *Calidris canutus islandica* breeds in high arctic Canada and winters in temperate coasts of western Europe, whereas *Calidris canutus canutus* breeds in high arctic Siberia and winters in hot and salty (sub)tropical coasts in West and South Africa (Piersma et al. 2005; Piersma 2007; Piersma and van Gils 2011). In the western Wadden Sea, the main wintering site for *C. canutus islandica*, the average salinity is typically about 28 ‰ (based on daily water salinity recorded throughout this experiment) and winter mean air temperature during the coldest month (February) is about 3 °C [based on monthly ambient temperature recorded over 29 years (see Vézina et al. 2006)]. At the Banc d'Arguin, the main wintering site for *C. canutus canutus*, the average salinity is about 40 ‰ [with values over 50 ‰ in some areas where strong evaporation occurs (Wolff and Smit 1990; Lavaud et al. 2013)] and the average air temperature is around 25 °C [with values over 40 °C during the daylight period (Wolff and Smit 1990)]. Because their osmoregulatory and thermoregulatory demands during the winter must differ widely, birds of these subspecies

might either show ecophysiological adaptations at the level of the subspecies, or ‘just’ show general flexible responses to environmental conditions (Piersma et al. 1996).

In this study, we exposed these two red knot subspecies, under controlled captive conditions, to different salinity–temperature combinations: high salinity (HS)–warm (40 ‰, 35 °C); HS–cold (40 ‰, 5 °C); LS–warm (28 ‰, 35 °C); and LS–cold (28 ‰, 5 °C). Such combinations can be encountered in the wild and should elicit different osmoregulatory (e.g. Gutiérrez et al. 2011, 2012) and thermoregulatory (e.g. Vézina et al. 2006) responses. To capture a broad picture of the physiological state and performance, we measured a suite of physiological and morphological parameters:

1. Food intake has profound implications for the relative intakes of water and salts, and thus, for an animal’s osmoregulatory responses (reviewed by Gutiérrez 2014).
2. The sizes of the gizzard and the salt glands are indicators of relative food intake (van Gils et al. 2003, 2005a, c) and concentrating ability (Staaland 1967; Gutiérrez et al. 2012), respectively.
3. Data on BMR, fat stores, body mass and temperature provide valuable information on an animal’s nutritional and energetic state (Butler and Woakes 2001; McNab 2002); also, changes in BMR may be linked to organ size because organs have high metabolic activities relative to other body structures (Hammond and Diamond 1997; Piersma et al. 2004).
4. Blood parameters such as levels of heat-shock proteins (HSPs; also known as ‘stress proteins’), leukocyte profiles [specifically the heterophil to lymphocyte (H/L) ratio], and natremia (plasma Na^+ concentration) are recognized as good indicators of stress.

HSPs are molecular chaperones that play key roles in the maintenance of cellular homeostasis under variable environmental conditions (reviewed by Sørensen et al. 2003). In particular HSP70 is considered to be the major HSP family in vertebrates and has been used as an index of a range of stressors including low/high temperatures and salinities (Sørensen et al. 2003). HSPs are not biased by handling stress and, therefore, they are an appropriate measure of short- and long-term stress (Herring and Gawlik 2007). The H/L ratio is positively related to the magnitude of the stressor and to the circulating glucocorticoids (reviewed by Davis et al. 2008; see also Müller et al. 2011). Natremia, an indicator of osmotic and ionic balance, should reflect the outcome of the osmotic challenges faced during the different salinity–temperature regimes (see Brischoux et al. 2013b, 2014).

We hypothesized that red knots will adjust physiological and morphological parameters as a function of their

osmoregulatory and thermoregulatory demands, and that those adjustments might vary within subspecies. On the basis of previous studies, we predict that adjustments (e.g. in BMR, food intake and body composition) would be more pronounced under more osmotically challenging conditions (i.e. HS–warm treatment). We also predict higher BMRs at HS (Gutiérrez et al. 2011) and cold (Vézina et al. 2006); reduced food intake, body mass and gizzard size (Vézina et al. 2006; Oudman et al. 2014), but enlarged salt glands (Gutiérrez et al. 2012), in birds exposed to HS–warm conditions. Second, if the physiological adjustments are partially fixed at the subspecies level, we would predict that tropically wintering *C. canutus canutus* deal with HS–warm better than temperate-wintering *C. canutus islandica*, i.e. they would regulate their natremia more precisely and show a weaker stress response (lower H/L ratio and HSP70 levels). Lastly, we would also predict that *C. canutus islandica* would show a weaker stress response under cold exposure than *C. canutus canutus*.

Materials and methods

Birds and housing

Twenty-one adult (non-moulting) red knots were used in this experiment. Of these, 11 individuals (five males, six females) belonged to the subspecies *C. canutus islandica* and ten individuals (seven males, three females) belonged to the subspecies *C. canutus canutus*. All birds were captured with mist nets in the western Dutch Wadden Sea (53°31′N, 6°23′E) in August–September 2013. Birds were aged according to plumage characteristics and sexed using molecular sexing (Baker et al. 1999). They were also assigned to one of the two subspecies co-occurring in the Wadden Sea during southward migration following Nebel et al. (2000). Afterwards, birds were brought into captivity at the Royal Netherlands Institute for Sea Research (NIOZ), Texel, the Netherlands, where they were housed in outdoor cages (4.5-m × 1.5-m surface × 2.5-m height) with unlimited access to water and trout pellets (see Vézina et al. 2006 for details). Each cage held four to six same-subspecies individuals throughout the experiment.

In January 2014, birds were randomly assigned to four indoor identical cages with the same dimensions as the outdoor cages. Then, the birds were fed a diet composed exclusively of 2 to 4 mm mud snails *Peringia ulvae* (formerly *Hydrobia ulvae*) collected by dredging in the Wadden Sea (as in Vézina et al. 2006). Mud snails were presented in two trays (60-cm × 40-cm surface × 5-cm height) with running seawater. This gastropod species is one of the main prey for red knots wintering in the Wadden Sea (van Gils et al. 2003, 2005b) and staging along southern European

coasts during migration (Moreira 1994). Because this prey is isosmotic to seawater (Todd 1964), red knots inevitably consume salt when they ingest them whole. After 2 weeks of acclimation to mud snails, which ensured that red knots had enough time to adjust to a diet of hard-shelled mollusc prey (Piersma et al. 1993), the four groups were exposed to four salinity-temperature combinations: HS–warm (40 ‰, 35 °C), HS–cold (40 ‰, 5 °C), LS–warm (28 ‰, 35 °C), and LS–cold (40 ‰, 5 °C). LS seawater was pumped directly from the sea, whereas HS levels were achieved by adding pure (99.9 %) refined salt (NaCl) to LS seawater. Each treatment lasted 2 weeks. Throughout, birds were held under a 12-h light:12-h dark photoperiod with no access to freshwater.

Experimental protocol

We employed a staggered design because it was logistically impossible to measure all the physiological parameters in all groups and individuals simultaneously. The choice of a 2-day time lag among groups (i.e. 6-day time lag between the first and the last group) allowed us to do so. On day 12, starting at 1000 hours, we began daily food-intake measurements, which were carried out over 2 consecutive days. On the last day of each treatment (days 14/15 starting at 1000 hours) we collected two to three birds from one cage for blood sampling, salt gland scoring and ultrasound measurements (see below). We then placed the birds in individual holding cages (0.60-m × 0.40-m surface × 0.30-m height) with access to saltwater (salinity of the corresponding treatment) but no food for about 5 h to create a post-absorptive condition prior the onset of the BMR measurements (see below). Prior to metabolic measurements, birds were weighed (to the nearest 0.1 g) and scored for the extent of their subcutaneous fat stores using a semiquantitative scale for shorebirds [0–7, with 7 being the fattest (Meissner 2009)]. We then measured BMR over a period lasting 17 h starting at 1600 hours. At the end of the metabolic trials temperature was monitored with an electronic temperature recorder (Omega 450 ATT; Stanford, CT) attached to a copper constantan thermocouple that was inserted 1.5 cm into the bird's cloaca. Afterwards, we measured body mass for the second time and moved the birds back to the cages to start the following treatment. A random treatment sequence was used. Water salinity and temperature were measured daily with a portable multi-parameter instrument (HD2156.1; Delta Ohm, Benelux). The experimental timeline is shown in Table 1.

Food and salt intake

We measured overall food intake in all the groups over 2 consecutive days in each treatment (as in Vézina et al.

Table 1 Experimental timeline designed to examine the effects of salinity and temperature on various physiological traits. The same procedure was repeated for the different salinity-temperature combinations using a 2-day time lag among groups

Time (hours)	Days 1–12	Day 13	Day 14	Day 15	Day 16
0800	Lights on	Lights on	Lights on	Lights on	Lights on
0900	–	–	–	Birds removed from the respirometer, measured for body temperature, weighed, and returned to their cage	Birds removed from the respirometer, measured for body temperature, weighed, and returned to their cage
0930	Temperature and salinity recordings	Temperature and salinity recordings	Temperature and salinity recordings	Temperature and salinity recordings	Temperature and salinity recordings
1000	Placement and withdrawal of food trays	Placement and withdrawal of food trays (FIR 1)	Placement and withdrawal of food trays (FIR 2)	Placement and withdrawal of food trays	Placement and withdrawal of food trays
1530	–	–	Two to three birds blood sampled and fasted for basal metabolic rate (BMR) measurements	Two to three remaining birds blood sampled and fasted for BMR measurements	Ultrasound measurements
1600	–	–	Previous birds weighed and salt gland/fat scored	Previous birds weighed and salt gland/fat scored	Birds moved to next treatment
2000	Lights off	Lights off	Previous birds placed in the respirometer	Previous birds placed in the respirometer	Lights off

2006). Every morning we sieved freshly thawed mud snails to remove all visible water and then took three subsamples (ca. 30 g) of food from this stock. Then we gave a pre-weighed amount of food from the same stock to the birds in a tray containing running saltwater (salinity depending on the treatment) at precisely 1000 hours. The following day, the food trays were removed from the cages at 1000 hours and the remaining food was carefully sieved to remove the water, and weighed again. The subsamples and leftover food were then dried for several days to constant mass in an oven at 60 °C. Following this, the dried samples were burned at 560 °C in a furnace for 5 h to obtain ash mass. Data are presented as the ash-free dry mass (AFDM) of mud snails consumed per bird over 24 h.

We additionally estimated salt (NaCl) concentration of dead mud snails provided as food in both LS and HS treatments using a modified protocol of Mahoney and Jehl (1985) and Blakey et al. (2006). To do this, we repeatedly blotted the surface water from duplicate samples weighing about 1 g each until we felt we could get no more water out without crushing the mud snails. Each sample was then mashed using a mortar, mixed with 0.4 ml Na⁺-free water, and centrifuged for 10 min at 4000g to separate the supernatant solution. A subsample of 30 µl was digested in 30-ml Teflon vials containing 1 ml of concentrated ultrapure HNO₃ and heated at 125 °C in a Savillex hot block during 48 h. After digestion, 3 ml of ultrapure water (MilliQ) was added and 1 ml of subsample was diluted with 9 ml of 0.1 M ultrapure HNO₃. An additional 10× dilution was necessary for determining Na⁺ concentration by inductively coupled plasma mass spectrometry (ICP-MS; Thermo Scientific iCAP Q ICP-MS; Thermo Fisher Scientific, Bremen). Na⁺ was then converted to salt intake assuming the 1:1.81 weight ratio of Na⁺ and Cl⁻ in seawater and osmoconforming marine invertebrates (e.g. Blakey et al. 2006). Salt intake rate (SIR; expressed as grams of salt consumed per bird over 24 h) was estimated for both LS and HS treatments by:

$$\text{SIR} = (\text{WM}_m \times \text{BW}_m \times S_m + \text{WM}_m \times \text{AD}_m \times S_t) \div 1000$$

where WM_m is the wet mass of consumed mud snails (in grams), BW_m is the body water fraction of wet mud snail samples (32.50 %), S_m is the salinity of mud snails (25.26 and 37.95 g kg⁻¹ in LS and HS, respectively), AD_m is the adherent water fraction on wet mud snail samples (26.91 %), and S_t is the salinity of each treatment (27.04 and 39.80 g kg⁻¹ in LS and HS, respectively). Note that this is a conservative estimate of salt intake since we did not consider salt ingested by drinking. However, daily focal observations made from behind a one-way mirror suggest that salt intake by drinking was negligible.

Blood sampling

Blood samples for leukocyte profiles, stress proteins and ions analyses were obtained by puncturing the alar vein using a 26-gauge needle and collecting blood (≈200 µl) into heparinized capillary tubes. For each individual and treatment, blood smears were obtained by transferring a small drop of blood from the capillary tube to a clean air-dried glass slide. The remaining blood was transferred to Eppendorf tubes which were centrifuged for 10 min at 4000g to separate plasma from cells and then stored at -20 °C until assay.

Leukocyte profiles

Blood smears were sent to the European Veterinary Laboratory, Woerden, the Netherlands, and examined by one person who was blind to the treatments. The slides were air-dried, fixed in absolute methanol and then stained with Wright Giemsa. The first 200 white blood cells per slide were identified and counted as lymphocytes, heterophils, basophils, monocytes or eosinophils. We then calculated the H/L ratio. A subsample of smears (*n* = 20) were scanned twice, and repeatability analyses (details below) showed that there was moderately good agreement between these two scans within individuals [heterophils, *R* = 0.67, 95 % credible intervals (CI) 0.40–0.93, *P* < 0.001; lymphocytes, *R* = 0.56, 95 % CI 0.24–0.89, *P* < 0.001; H/L ratio, *R* = 0.61, 95 % CI 0.31–0.91, *P* < 0.001].

Heat-shock proteins

We determined HSP70 in blood cells as in Herring et al. (2011) with some modifications. Red blood cells were washed three times with phosphate-buffered saline and after centrifugation the supernatant was aspirated from the final wash and mixed with ×1 extraction reagent and protease inhibitor cocktail (Sigma Aldrich, Spain). Samples were sonicated for 1 min and incubated on ice for 30 min. with occasional mixing. Then, samples were centrifuged again (10 min, 21,000g, 4 °C) and the supernatant was removed and used in posterior analyses. Cell lysates total protein concentration was determined by the Bradford method using bovine serum albumin as the standard. Concentrations of HSP70 were determined from the cell lysates by means of an enzyme-linked immunosorbent assay kit specific for inducible HSP70 (HSP70 EIA kit; Enzo Life Sciences, Lausen, Switzerland). All samples were run in duplicate and the means of duplicates were used in all analyses. All final HSP70 values were standardized by dividing HSP70 concentration by total protein to facilitate comparisons. Based on internal standards run in duplicate, the

mean intra- and inter-assay coefficients of variation were 4.1 and 6.9 %, respectively.

Plasma Na^+ concentrations

Plasma Na^+ concentrations were determined using an electrolyte analyser with ion-specific electrodes (SPOTCHEM EL SE-1510; Menarini, Milan). Briefly, we allowed stored plasma samples and exclusive E-plates to reach room temperature (15–20 °C), stirred the samples without foaming, and then distributed subsamples (22 μl) on the E-plates before starting the automated measurement.

Basal metabolic rate

Measurements of basal rates of O_2 consumption and CO_2 production were taken from each individual at the end of each treatment using open-flow respirometry. For details about the respirometry set-up and BMR measurement protocol, see Piersma et al. (2004). Briefly, birds were measured overnight under post-absorptive digestive conditions in dark metabolic chambers at 21.0 °C (± 0.1), i.e. within the red knot's thermoneutral zone (Piersma et al. 1995). BMR was defined as the lowest 10-min average of O_2 consumption, which was calculated with the appropriate formulas for our set-up (Piersma et al. 2004).

Salt gland scores

We did not use ultrasonography for estimating salt gland size since the head is a uniformly heavily feathered area and the air in the feathers limits the use of such a technique (Dietz et al. 1999). Instead, we estimated salt gland size for each individual using a novel non-invasive approach (Gutiérrez 2014). We used sensory evaluation to score the thickness of the salt glands at the postorbital ridge. This consisted of sliding a finger across a smooth polyvinyl-chloride plate prepared with five increasing thicknesses (0–0.8 mm; artificially created by overlapping 0.16-mm-thick tape portions) at regular distances from each other, and then comparing these thicknesses with those of the postorbital salt gland ridge. Two trained observers (J. S. G. and A. S.-R.) blind to the treatments independently scored the salt gland ridge on an 11-point ordinal scale (0–5, including half-points). Repeatability analyses (details below) showed that there was good agreement between observers ($R = 0.71$ – 0.78 , always $P < 0.001$), so we used the mean of the two scores for the subsequent analyses. Glandular tissue can vary in height and width but rarely in length (Staal and 1967; Siegel-Causey 1990); then, the thickness (i.e. height) of the saltglands at the postorbital ridge might be used a proxy of saltgland size.

Ultrasonography

Measurements of the gizzard dimensions (height and width) were done in duplicate by one observer (A. D.) with a Pie 200 ultrasound instrument with a 7.5-MHz linear probe (Pie Medical Benelux, BBV, Maastricht, the Netherlands) as described by Dietz et al. (1999). Prior to the experiment, calibration curves were made for the observer using 20 dead red knots with a wide range of gizzard sizes. As the repeatabilities between the first and the second measurements were high (gizzard height, $R = 0.90$ – 0.96 , always $P < 0.001$; gizzard width, $R = 0.73$ – 0.95 , always $P < 0.001$), we used the mean values for statistical analyses. The gizzard dimensions were converted to gizzard masses as follows:

$$\text{Gizzard mass (g)} = -1.09 + 3.78 \times (\text{gizzard height} \times \text{gizzard width}).$$

Statistical analyses

Analyses were performed using R version 3.1.0 statistical software (R Development Core Team). Throughout, we used $P < 0.05$ as the level of significance. Data are presented as mean \pm SEM.

We fitted linear mixed-effects models [package nlme (Pinheiro et al. 2014)] with each parameter (food and salt intake, BMR, body mass, body temperature, fat and salt gland scores, gizzard mass, HSP70, H/L ratio and Na^+) as the response variable; salinity, temperature and subspecies as fixed factors; and group and individual nested within group as random effects (except for food and salt intake where group values are reported). Low sample sizes did not allow a comparison by sex; however, earlier studies did not find sex-dependent differences in various physiological traits, including total body mass and fat stores (Piersma et al. 1999), stress levels (Reneerkens et al. 2002) and immune function (Buehler et al. 2009a, b). We always started with the full model and simplified it using backwards elimination based on ANOVA with $P < 0.05$ as the selection criterion until reaching the minimal adequate model. Model assumptions were checked using the residuals of the final model. For those traits measured in duplicate for each salinity-temperature regime, we calculated ANOVA-based repeatabilities (the intraclass correlation coefficient) using the package rptR [rpt.aov function (Nakagawa and Schielzeth 2010)]. Three individuals (two *C. canutus canutus* and one *C. canutus islandica*) did not adjust to the combination of HS and high temperature (body mass below the critical threshold of 100 g) so they were returned to outdoor aviaries with unlimited access to freshwater and trout pellets and excluded from further analysis. The sample size for HSP70 differs from that for other response variables because the HSP70 concentration in 12

Table 2 Statistics and coefficients of minimum adequate models for food and salt intake in two subspecies of red knot acclimated to different salinity-temperature combinations

Response variables	Predictors	Coefficients	SEM	df	t-value	P-value
Food intake (g AFDM)	<i>Intercept</i>	32.79	2.01	24	16.35	<0.001
	Salinity ^a	0.00	2.55	24	0.00	0.999
	Temperature ^b	−17.99	2.55	24	−7.06	<0.001
	Subspecies ^c	−1.07	1.77	2	−0.61	0.606
	Salinity × temperature	13.23	3.54	24	3.74	0.001
Salt intake (g)	<i>Intercept</i>	8.67	0.37	24	23.71	<0.001
	Salinity ^a	−2.68	0.37	24	−5.35	<0.001
	Temperature ^b	−5.51	0.50	24	−11.01	<0.001
	Salinity × temperature	3.84	0.70	24	5.51	<0.001

Significant terms are depicted in *italic*

AFDM Ash-free dry mass

^a Reference category is 'high'

^b Reference category is 'cold'

^c Reference category is '*Calidris canutus canutus*'

samples was below the detection threshold, independently of the treatment and subspecies. Three extreme outliers (>3 SD) for this response variable were considered as missing values.

Results

Baseline values, measured prior to the experimental conditions, did not differ significantly between subspecies in any of the 11 parameters measured (always $P > 0.05$). Therefore, actual values for each subspecies were used in all analyses. Because BMR was significantly correlated with body mass (linear regression, $F_{1,74} = 50.44$, $P < 0.001$), we also used residuals of the body mass regressions for this variable in subsequent analyses.

Responses in food and salt intake

Temperature affected food intake rate with birds acclimated to the cold eating on average 35 % more food (32.29 ± 1.14 g AFDM bird^{−1} day^{−1}) than individuals from the warm treatments (20.89 ± 2.11 g AFDM bird^{−1} day^{−1}), independently of salinity and subspecies (Table 2; Fig. 1a). We found no significant effect of salinity (Table 2), although birds of both subspecies substantially reduced food intake rates under HS-warm conditions (Fig. 1a). As a result, the interaction term salinity × temperature, but not subspecies, was highly significant (Table 2). Interestingly, the food intake rate of warm-acclimated birds was

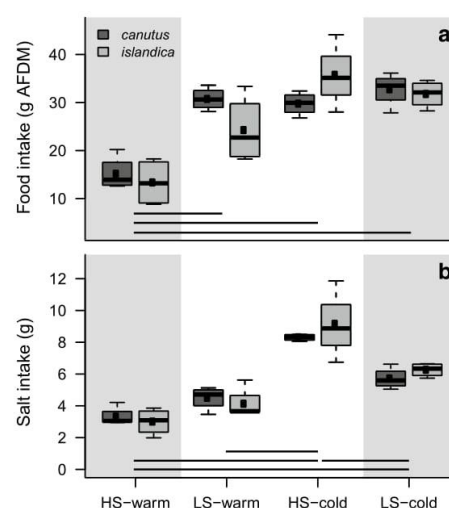


Fig. 1 Food (a) and salt (b) intakes in two red knot subspecies acclimated to different salinity-temperature combinations. *Box-and-whisker plots* give means estimated by the full model (large filled square), medians (horizontal line within plot), interquartile ranges (box), and ranges (bars). *Shaded areas* depict wintering salinity-temperature conditions potentially experienced by red knots in West Africa (*Calidris canutus canutus*) and Western Europe (*Calidris canutus islandica*), respectively. Group means that are statistically significantly different from each other (at the 5 % confidence level as indicated by post hoc Tukey tests) are connected by horizontal bars (subspecies did not differ within treatments in any case, so we did not include it in the tests). Note that these two variables represent group-specific measurements. HS High salinity, LS low salinity

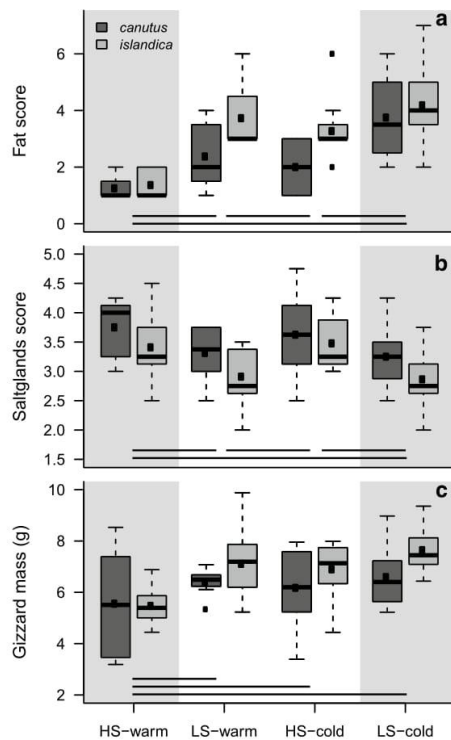


Fig. 2 Fat score (a), salt-gland score (b), and gizzard mass (c) in two red knot subspecies acclimated to different salinity-temperature combinations. See Fig. 1 for more details and abbreviations

on average 55 % lower when they fed at elevated salinity (14.27 ± 1.45 g AFDM bird⁻¹ day⁻¹ for HS-warm and 27.50 ± 2.10 g AFDM bird⁻¹ day⁻¹ for LS-warm).

Salinity, temperature and their interaction significantly affected SIR (Table 2). Birds exposed to HS-warm showed the lowest SIRs (3.16 ± 0.24 g NaCl bird⁻¹ day⁻¹), whereas those exposed to HS-cold showed the highest SIRs (8.67 ± 0.59 g NaCl bird⁻¹ day⁻¹) (Fig. 1b). Regardless of the treatment, both subspecies exhibited similar SIRs (subspecies, $P = 0.704$; Fig. 1b).

Responses in fat stores and digestive organs

Fat score and body mass were similarly affected by explanatory variables (Figs. 2a, 3a; Tables 3, 4, respectively), since they were highly correlated with each other (linear regression, $F_{1,74} = 451.5$, $P < 0.001$, subspecies and treatments combined). For fat score, salinity and the three-way interaction (salinity \times temperature \times subspecies) were significant (Table 3). HS-acclimated birds had lower fat score,

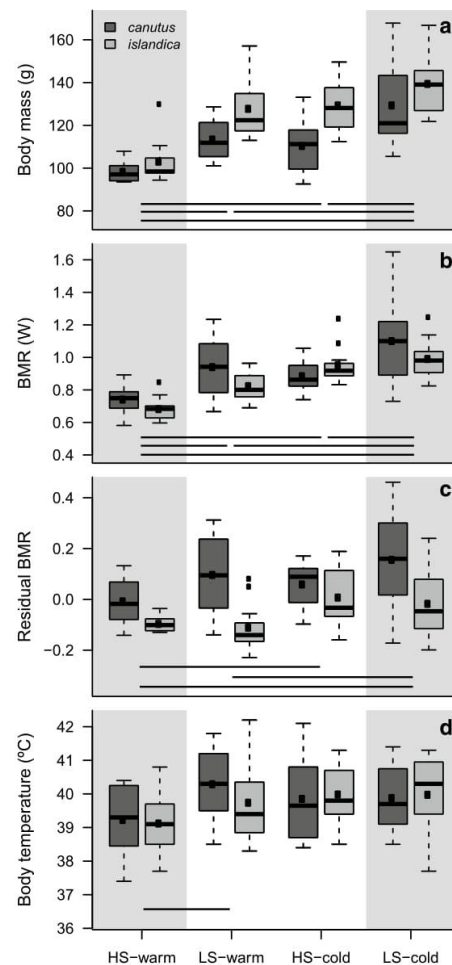


Fig. 3 Body mass (a), basal metabolic rate (BMR) (b), residual BMR (c), and body temperature (d) in two red knot subspecies acclimated to different salinity-temperature combinations. See Fig. 1 for more details and other abbreviations

especially under warm conditions (Fig. 2a). At HS-cold, *C. canutus canutus* exhibited lower fat scores than *C. canutus islandica* (Fig. 2a).

Salt gland score (an indicator of concentrating ability) was affected by salinity only (Table 2). Salt gland scores were higher under the HS conditions, regardless of temperature and subspecies (Fig. 2b; Table 3). Overall, salt gland scores were positively correlated with mass-corrected (i.e. residual) BMR (linear regression, $F_{1,74} = 5.58$, $P = 0.021$) and negatively correlated with body mass (linear regression, $F_{1,74} = 11.87$, $P < 0.001$). The variation in gizzard

Table 3 Statistics and coefficients of minimum adequate models for morphological parameters measured in two subspecies of red knot acclimated to different salinity–temperature combinations

Response variables	Predictors	Coefficients	SEM	df	t-value	P-value
Fat score (0–7)	<i>Intercept</i>	2.00	0.44	51	5.05	<0.001
	<i>Salinity</i> ^a	1.75	0.44	51	4.01	<0.001
	<i>Temperature</i> ^b	−0.75	0.44	51	−1.72	0.091
	<i>Subspecies</i> ^c	1.27	0.52	2	2.44	0.135
	<i>Salinity × temperature</i>	−0.63	0.62	51	−1.01	0.315
	<i>Salinity × subspecies</i>	−0.84	0.57	51	−1.47	0.148
	<i>Temperature × subspecies</i>	−1.16	0.57	51	−2.02	0.048
	<i>Salinity × temperature × subspecies</i>	2.08	0.81	51	2.57	0.013
Salt gland score (0–5)	<i>Intercept</i>	3.71	0.16	55	23.84	<0.001
	<i>Salinity</i> ^a	−0.49	0.09	55	−5.73	<0.001
	<i>Temperature</i> ^b	0.03	0.09	55	0.38	0.704
	<i>Subspecies</i> ^c	−0.32	0.19	2	−1.70	0.232
Gizzard mass (g)	<i>Intercept</i>	6.07	0.29	55	20.65	<0.001
	<i>Salinity</i> ^a	0.96	0.28	55	3.40	0.001
	<i>Temperature</i> ^b	−0.74	0.28	55	−2.62	0.011
	<i>Subspecies</i> ^c	0.60	0.28	2	2.10	0.171

Significant terms are depicted in *italic*^a Reference category is ‘high’^b Reference category is ‘cold’^c Reference category is ‘*C. canutus canutus*’**Table 4** Statistics and coefficients of minimum adequate models for physiological parameters measured in two subspecies of red knot acclimated to different salinity–temperature combinations

Response variables	Predictors	Coefficients	SEM	df	t-value	P-value
Body mass (g)	<i>Intercept</i>	112.65	4.02	55	28.01	<0.001
	<i>Salinity</i> ^a	17.22	2.28	55	7.55	<0.001
	<i>Temperature</i> ^b	−16.88	2.28	55	−7.39	<0.001
	<i>Subspecies</i> ^c	12.01	4.84	17	2.48	0.024
BMR (W)	<i>Intercept</i>	0.91	0.05	54	19.78	<0.001
	<i>Salinity</i> ^a	0.18	0.04	54	6.02	<0.001
	<i>Temperature</i> ^b	−0.22	0.03	54	−8.49	<0.001
	<i>Subspecies</i> ^c	0.00	0.06	2	0.08	0.945
Residual BMR	<i>Salinity × subspecies</i>	−0.12	0.05	54	−2.60	0.012
	<i>Intercept</i>	0.07	0.04	54	1.69	0.097
	<i>Salinity</i> ^a	0.10	0.03	54	3.98	<0.001
	<i>Temperature</i> ^b	−0.08	0.02	54	−5.11	<0.001
	<i>Subspecies</i> ^c	−0.07	0.05	2	−1.37	0.303
Body temperature (°C)	<i>Salinity × subspecies</i>	−0.12	0.03	54	−3.63	<0.001
	<i>Intercept</i>	39.79	0.30	55	132.13	<0.001
	<i>Salinity</i> ^a	0.41	0.22	55	1.85	0.070
	<i>Temperature</i> ^b	−0.36	0.22	55	−1.65	0.103
	<i>Subspecies</i> ^c	−0.11	0.34	2	−0.32	0.782

Significant terms are depicted in *italic*^a Reference category is ‘high’^b Reference category is ‘cold’^c Reference category is ‘*C. canutus canutus*’

mass (an indicator of relative food intake) was affected by salinity and temperature, but not subspecies (Table 3). HS–warm-acclimated birds showed the smallest gizzards

(5.51 ± 0.26 g), whereas LS–cold-acclimated birds showed the largest gizzards (7.20 ± 0.32 g) (Fig. 2c). Gizzard mass positively correlated with whole-organism BMR (linear

Table 5 Statistics and coefficients of minimum adequate models for blood parameters measured in two subspecies of red knot acclimated to different salinity-temperature combinations

Response variables	Predictors	Coefficients	SEM	df	t-value	P-value
HSP70 (ng mg ⁻¹)	Intercept	0.28	0.16	40	1.78	0.082
	Salinity ^a	0.17	0.21	40	1.23	0.226
	Subspecies ^c	0.21	0.22	2	0.97	0.432
	Salinity × subspecies	-0.43	0.19	40	-2.27	0.029
H/L ratio	Intercept	1.60	0.24	53	6.69	<0.001
	Salinity ^a	-0.54	0.21	53	-2.54	0.014
	Temperature ^b	0.03	0.21	53	0.14	0.887
	Subspecies ^c	-1.10	0.33	2	-3.39	0.077
	Salinity × subspecies	0.60	0.28	53	2.15	0.036
	Temperature × subspecies	0.51	0.28	53	1.84	0.072
	Salinity × temperature	0.51	0.28	53	1.84	0.072
Na ⁺ (mmol L ⁻¹)	Intercept	164.01	2.33	53	70.32	<0.001
	Salinity ^a	-7.56	3.23	53	-2.34	0.023
	Temperature ^b	9.74	2.48	53	3.92	<0.001
	Subspecies ^c	-5.47	2.59	2	-2.11	0.170
	Salinity × temperature	-9.26	3.51	53	-2.66	0.011
	Salinity × subspecies	11.96	3.56	53	3.36	0.001

Significant terms are depicted in *italic*

HSP70 Heat-shock protein 70, H/L heterophils/lymphocytes

^a Reference category is 'high'^b Reference category is 'cold'^c Reference category is '*C. canutus canutus*'

regression, $F_{1,74} = 14.02$, $P < 0.001$), but not with mass-corrected BMR (linear regression, $F_{1,74} = 0.08$, $P = 0.776$). We found no correlation between salt gland scores and gizzard mass either when data were pooled (linear regression, $F_{1,74} = 3.14$, $P = 0.081$) or when data were analysed for each of the treatments separately (always $P > 0.123$).

Responses in body mass, basal metabolism and temperature

Body mass decreased with salinity and temperature in both subspecies (Fig. 3a). Regardless of the salinity and temperature, *C. canutus islandica* overall exhibited higher body masses than *C. canutus canutus* (Fig. 3a; Table 4).

Both whole-organism and mass-corrected BMR were affected by salinity and temperature (Table 4). Overall, BMR tended to be higher in cold treatments (Table 4; Fig. 3b, c). The salinity × subspecies interaction was also significant for both whole-organism and residual BMR (Table 4), indicating that *C. canutus canutus* had higher levels under low salinities.

None of the explanatory variables significantly affected body temperature (Table 4; Fig. 3d). We found no relationship between body temperature and body mass or fat scores (linear regressions, $F_{1,74} = 1.73$, $P = 0.19$; $F_{1,74} = 0.67$, $P = 0.41$, respectively). However, body temperature positively correlated with both whole-organism (linear

regression, $F_{1,74} = 10.39$, $P = 0.002$) and mass-corrected BMR (linear regression, $F_{1,74} = 9.074$, $P = 0.004$).

Responses in blood parameters

We found no direct effect of temperature, salinity and subspecies on HSP70 (Table 5; Fig. 4a), although HS-exposed birds had somewhat higher levels of HSP70 (0.39 ± 0.09 ng mg⁻¹) than LS-exposed ones (0.35 ± 0.06 ng mg⁻¹). There was a significant interaction between salinity and subspecies such that *C. canutus islandica* had higher HSP70 levels than *C. canutus canutus* in HS but lower levels in LS (Table 5; Fig. 4a). The variation in H/L ratios was affected by salinity with HS-exposed knots having higher ratios than LS-exposed ones (1.12 ± 0.13 vs. 0.93 ± 0.10) (Table 5; Fig. 4b). The significant interaction between salinity and subspecies indicated that *C. canutus canutus* had higher ratios in HS treatments (Table 5; Fig. 4b). H/L ratio and HSP70 levels were not significantly correlated (linear regression, $F_{1,62} = 2.43$, $P = 0.123$).

Salinity and its interaction with subspecies affected plasma Na⁺ levels (Table 5). Plasma Na⁺ increased with salinity, with HS-warm-acclimated birds showing the highest levels. Under such conditions, *C. canutus canutus* exhibited higher Na⁺ levels (173.25 ± 4.74 mmol L⁻¹) than *C. canutus islandica* knots (168.64 ± 2.90 mmol L⁻¹).

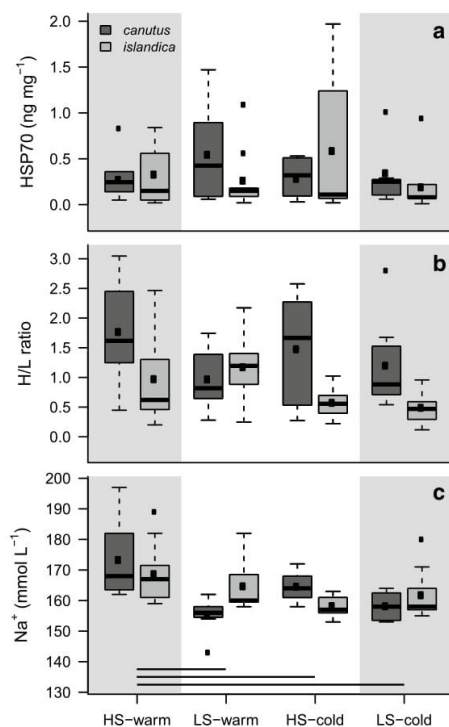


Fig. 4 Heat-shock protein 70 (HSP70) (a), heterophils/lymphocytes (H/L) ratio (b), and plasma Na⁺ (c) in two red knot subspecies acclimated to different salinity-temperature combinations. See Fig. 1 legend for more details and other abbreviations

(Fig. 4c). Na⁺ level was not correlated with H/L ratio (linear regression, $F_{1,74} = 2.43$, $P = 0.123$) or HSP70 (linear regression, $F_{1,74} = 0.04$, $P = 0.85$).

Discussion

In the present study we investigated the physiological adjustments to salinity and temperature in two red knot subspecies with contrasting non-breeding osmoregulatory demands. We found that the simultaneous demands of osmoregulation and thermoregulation yielded changes in energy acquisition and allocation as reflected in phenotypic flexibility of food and salt intake, basal metabolism, body composition and stress responses. Importantly, our results demonstrate that, regardless of the subspecies, the combination of relatively high salinities and temperatures (naturally encountered by shorebirds and other migratory waterbirds) limited the rate of food (energy) acquisition with direct and indirect consequences for the size of metabolically active organs. As a consequence, birds coping with

HS-warm conditions showed several significantly reduced physiological and condition-related traits (body mass, fat stores, gizzard mass and BMR) and augmented stress-related traits (HSP70, H/L ratio and plasma Na⁺).

It is well established that salt loads can have a direct negative effect on the physiology of birds that inhabit saline environments (Sabat 2000; Gutiérrez 2014). Previous studies have shown that salt intake can constrain food intake in captive red knots (Klaassen and Ens 1990; Oudman et al. 2014). In this study we found that red knots feeding on HS mud snails under warm conditions extensively reduced food and energy intake rate. Assuming that 40 % of *Peringia* AFDM constitutes indigestible ballast material (Quaintance et al. 2011), an energy density of 22 kJ g⁻¹ AFDM (Zwarts and Wanink 1993) and an assimilation efficiency of 80 % (Kersten and Piersma 1987), the metabolisable energy intake rate of HS-warm-exposed red knots was about 136 kJ day⁻¹, in contrast with the 290 kJ day⁻¹ consumed by LS-warm-exposed ones. These crude estimates represent only a quarter to a half of the upper limit of daily metabolizable energy for a red knot feeding for 12 h day⁻¹ [544 kJ day⁻¹ (van Gils et al. 2005a)], which suggests that managing salt and heat loads may pose a major osmoregulatory challenge for tropical-wintering shorebirds feeding on poor-quality molluscs. This could partly explain why red knots at tropical intertidal sites have lower fuelling rates than birds at higher latitudes (Piersma et al. 2005). Daily food intake, and hence fuelling rates, may be constrained by heat production due to external heat loads in the tropics (Verboven and Piersma 1995; Battley et al. 2003), but also by additional salt loads (Klaassen and Ens 1990; Verboven and Piersma 1995). Our results clearly show that fuelling rates are likely to be constrained by the combination thereof.

Likewise SIR is reduced in warm-acclimated birds, even at relatively low salinities. This suggests that in hyperosmotic environments food/salt intake is probably limited by evaporative water loss rather than by the total amount of ingested salt. That is, water loss could exceed salt gain resulting in water imbalance. Surprisingly, the direct salt intake in captive knots reached up to 9 g day⁻¹ leading to a mass-specific salt intake of 71 mg g⁻¹ body mass. This mass-specific salt intake is much higher than reported for other marine birds, including that of wintering common eiders *Somateria mollissima* feeding on whole-shelled bivalves [24 mg g⁻¹ (Nelhs 1996)]. Assuming the overall cost of salt turnover at 1.3 kJ g⁻¹ NaCl (Nelhs 1996), HS-cold-acclimated red knots would demand ca. 11 kJ day⁻¹ to eliminate excess salt, which represents approximately 14 % of their BMR. Such a figure is comparable to the energetic cost of osmoregulation and other physiological demands in waterbirds and probably sufficient to trade-off with other physiological demands (Gutiérrez 2014).

Arguably the experimental conditions described here do not always very closely resemble conditions in the field. However, these results can be used as a proxy for comparative examinations of salt tolerance under different thermal environments. Although our experimental birds were acclimated to the different treatments over relatively short time periods, the fact that three individuals did not adjust to the HS–warm treatment and that they all continued to lose mass under such conditions suggests that we may have come close to their maximum salt tolerance. It is worthwhile to note that birds were exposed to a constant elevated temperature under windless aviary conditions which did not allow them to benefit from the lower night temperatures and the sea breeze they normally experience at tropical intertidal sites. In this sense, it is possible that shorebirds fuelling at the tropical sites avoid heat stress by feeding at night (Zwarts et al. 1990a), and by means of evaporative cooling and cutaneous evaporation (Battley et al. 2003).

The decrease in food intake was accompanied by a reduction in BMR when red knots were simultaneously exposed to HS and heat. This supports earlier findings that red knots downregulate BMR in response to heat acclimation/acclimatization over short periods (e.g. Piersma et al. 1995; Kersten and Piersma 1987; Vézina et al. 2006). However, BMR did not increase with salinity as previously shown in a closely related species (Gutiérrez et al. 2011), possibly because salinity and temperature generated opposite responses in basal metabolism (e.g. Vézina et al. 2006; Gutiérrez et al. 2011), making it impossible to disentangle the effects of the two factors. Nevertheless, birds with higher salt gland scores showed higher BMR after accounting for differences in body mass, indicating that when exposed to saltier conditions birds do pay an additional energetic cost for osmoregulation.

We found no substantial changes in body temperature throughout the experiment. A slight daily or seasonal decrease in body temperature (facultative hypothermia) is shown in many avian species in response to stressful and energy-limiting conditions [e.g. breeding, migratory fuelling, low food supply and low temperature (Butler and Woakes 2001; McKechnie and Lovegrove 2002)]. Although birds exposed to warmth and HS tended to have lower body temperature, it did not differ significantly among the four treatments. Nevertheless, this should be interpreted cautiously since our birds were measured at the end of the metabolic trials, during which they were resting overnight under thermally neutral conditions and thus were not directly exposed to the experimental conditions in the acclimated aviaries. Overall, body temperature was positively correlated with BMR, indicating that individuals modulated these two traits in parallel. The decline of BMR and body temperature during prolonged heat exposure could have prevented the birds from overheating (Kersten and Piersma 1987; Klaassen 1990).

Based on increased HSP70 levels and H/L ratio, it seems that experimental red knots experienced the combination of HS and heat as stressful, resulting in an energy-saving strategy that reduced their food intake rate and BMR. Such metabolic downregulation was also paralleled by a reduction in gizzard mass, suggesting that differences in the size of several digestive organs of red knots could be correlated with differences in BMR (Piersma et al. 1996). In contrast, salt glands measurably increased in size with higher salinity at both high and low temperatures, which suggests that red knots require large salt glands to meet increased osmoregulatory demands (Gutiérrez et al. 2012), despite low food intake. Because salt gland hypertrophy in saline-acclimated birds can occur over short time scales (Peaker and Linzell 1975), it is likely that the size of the salt glands had reached a maximum after 2 weeks of HS acclimation and thus we could not detect differences associated with ambient temperature (see Gutiérrez et al. 2012).

It is interesting to note that red knots, especially those of the subspecies *C. canutus canutus*, exposed to HS showed elevated plasma Na^+ (up to 197 mmol L^{-1}). One might interpret this as an indication of either osmotic stress or a certain tolerance to hypernatremia over short time periods. The lack of correlation between Na^+ and stress indicators (HSP70 and H/L ratio) supports the latter interpretation. Indeed, the elevated natremia we recorded in captive red knots is within the range of values reported for other bird species with salt glands [$135\text{--}216 \text{ mmol L}^{-1}$ (Skadhauge 1981)]. Recent studies that reported elevated natremia in sea snakes (Brischoux et al. 2013b, 2014) suggested that greater tolerance to hypernatremia would be advantageous since active salt excretion would occur only when plasma Na^+ dangerously exceeds an upper threshold and this, in turn, would substantially decrease energetic costs associated with salt gland functioning. Whether *C. canutus canutus* could have developed a certain degree of tolerance to hypernatremia remains to be further explored. Moreover, the fact that *C. canutus canutus* had somewhat greater H/L ratios and lower body masses and fat scores when exposed to HS provides an interesting avenue for exploring possible differences at the subspecies level. For instance, *C. canutus islandica* held in captivity maintained the schedules of moult and body mass changes of their counterparts in the wild, whereas *C. canutus canutus* showed deviations from normal annual rhythms (Piersma et al. 1996; Piersma and Ramenofsky 1998). Experimental and observational evidence (Piersma et al. 1996; Piersma and Ramenofsky 1998) from red knots that have been kept for research purposes under long-term captive conditions at research facilities at northern temperate latitudes suggests that *C. canutus canutus* are more susceptible to stress than *C. canutus islandica*. One is then tempted to pose the question: do *C. canutus canutus* ‘wintering’ in such an aviary environment

somehow feel that they are at the ‘wrong’ latitude? Birds from different latitudes and with different migratory habits could, for example, have different photosensitive phases (e.g. Berthold 1974; Gwinner and Scheuerlein 1999). Further experiments under photoperiod and temperature regimes resembling those of the red knots’ natural environments are necessary to identify the reason(s) for such sub-specific differences.

In addition to the physiological mechanisms examined here, behavioural strategies leading to a decrease in salt intake are likely crucial in maintaining the osmotic balance (Gutiérrez 2014). Selective feeding on low-salt-load prey is a viable osmoregulatory strategy to avoid salt stress in saline environments (e.g. Purdue and Haines 1977; Nyström and Pehrsson 1988). For example, red knots staging on intertidal areas during the spring migration do not feed extensively on the crustacean brine shrimp *Artemia* spp. at supratidal salinas (100–150 ‰), an observation that has been explained by the avoidance of salt stress (Masero 2002). Instead, the mud snail (the prey used in this study) is the main prey for red knots staging on the mudflats of southern European coasts (Moreira 1994; Masero 2002). Feeding on less salty mud snails—despite their relatively low energy content (van Gils et al. 2005a)—might enable red knots to overcome osmotic stress.

A better understanding of avian adaptations and tolerances to saline environments is important to both basic science and conservation (reviewed in Gutiérrez 2014). In view of the fact that rather inactive red knots exposed to HS and temperature in captivity appeared challenged to even maintain body mass, it remains a big puzzle how hard-working birds in the field that have to commute between feeding and resting areas and may be forced into a lot of evasive flying by aerial predators (see Leyrer et al. 2012; van den Hout et al. 2014) can ever deposit the stores for onward flight in a place like Banc d’Arguin (Zwarts et al. 1990b; Piersma et al. 2005). Such knowledge is rather relevant at a time when fluctuating temperatures and salinity conditions predicted under various climate change scenarios (reviewed in Gutiérrez 2014) will affect the options open to birds and other aquatic air-breathing vertebrates such as sea turtles, snakes, and mammals.

Author contribution statement This study was conceived and designed by J. S. G., A. D., J. A. M. and T. P. The experiment was performed by J. S. G., A. S.-R. and A. D. HSP were measured by A. V. Data analyses were performed by J. S. G. and A. S.-R. The manuscript was written by J. S. G., J. A. M., A. V. and T. P.

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Relative abundance and distribution of fisheries influence risk of seabird bycatch

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Fisheries provide an abundant and predictable food source for many pelagic seabirds through discards, but also pose a major threat to them through bycatch, threatening their populations worldwide. The reform of the European Common Fisheries Policy (CFP), which intends to ban discards through the landing obligation of all catches, may force seabirds to seek alternative food sources, such as baited hooks from longlines, increasing bycatch rates. To test this hypothesis we performed a combined analysis of seabird-fishery interactions using as a model Scopoli's shearwaters *Calonectris diomedea* in the Mediterranean. Tracking data showed that the probability of shearwaters attending longliners increased exponentially with a decreasing density of trawlers. On-board observations and mortality events corroborated this result: the probability of birds attending longliners increased 4% per each trawler leaving the longliner proximity and bird mortality increased tenfold when trawlers were not operating. Therefore, the implementation of the landing obligation in EU waters will likely cause a substantial increase in bycatch rates in longliners, at least in the short-term, due to birds switching from trawlers to longliners. Thus the implementation of the landing obligation must be carefully monitored and counterbalanced with an urgent implementation of bycatch mitigation measures in the longline fleet.

Effects of fishing on marine megafauna are widespread and diverse, mainly due to overfishing, production of discards and bycatch^{1–3}. Bycatch, the incidental capture of non-target species, is of particular concern for long-lived species with low reproductive rates and delayed sexual maturity, such as seabirds⁴. Baited hooks offer the opportunity for an easy meal, yet these entail a very high risk of birds being hooked and subsequently drowned. A recent global review estimated seabird bycatch by longlines in 160,000–320,000 birds per year⁴. In fact, for some species, the current rates of bycatch are unsustainable for their long-term viability^{5–8}.

On the other hand, fishery discards may also have profound impacts on the breeding biology, distribution and population dynamics of seabirds, by making available demersal and benthonic species otherwise naturally inaccessible^{9–11}. Worldwide discards are estimated to be 8% of the total catch (i.e. around 7,000,000 tonnes discarded annually²). Ultimately, discards seem to be responsible for the increases in population sizes of several scavenging species over the last decades, such as large gulls^{9,12}.

Seabird-fishery interactions are of particular concern in the Mediterranean¹³; an enclosed and low-productive sea with a high degree of endemism¹⁴. Four seabird species are endemic to the basin and commonly caught by fishing gear, particularly in longlines: the Audouin's gull *Larus audouinii*, and the Scopoli's *Calonectris diomedea*, Yelkouan *Puffinus yelkouan* and Balearic shearwaters *Puffinus mauretanicus*^{15,16}. Among these, the Yelkouan and Balearic shearwaters are globally threatened (Vulnerable and Critically Endangered, respectively¹⁷). According to conservative estimates, at least 5,000 birds could be killed annually in the region¹⁸. In particular, the bycatch of Scopoli's shearwaters represents over 50% of all birds caught in longlines in some areas of the Western Mediterranean, which would imply that 4–6% of the local population breeding in the Balearic Islands is killed every year^{4,19,20}. For these species, bycatch by other gears appears to be far less relevant in the region^{15,16}.

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In the Mediterranean Sea, discards are estimated to be 18% of the catch (i.e. around 230,000 tonnes discarded annually), with trawlers being responsible for 15 to 65% of the discards¹⁸. Discard availability modifies the diet, foraging strategies and distribution of seabirds^{21–24}, with some species obtaining up to 75% of their energy needs from this resource^{25,13}.

To further complicate matters, interactions among different fisheries may lead to unexpected indirect effects on seabirds. Some recent studies in the Mediterranean provided evidence that the attendance of Scopoli's shearwaters to trawlers and longliners may depend on the relative activity schedules of these fleets^{26–28}. When trawlers do not operate, shearwaters may seek alternatives to discards, such as baits used by longline fisheries, with a consequent increase in the risk of being hooked^{27,28}. This possibility needs to be fully explored, since changes in fishery schedules or discard availability can occur at any time, and a proper management may minimize their negative impacts on seabird mortality. Indeed, the reform of the Common Fisheries Policy (CFP; <http://ec.europa.eu/fisheries/cfp/>) by the European Union (EU), among other measures, is implementing the elimination of discards through the so called *landing obligation*, with the aim of reducing the impact of fisheries on marine ecosystems. Therefore, there will be a gradual reduction in discards, from now to 2019, that could severely affect the Mediterranean seabird community, including the threatened shearwater species¹³. Thus, there is an urgent need to improve our understanding about the undesirable interactions among different fishery activities on seabird mortality.

To cast light on this problem, we studied the interaction between Scopoli's shearwater and fishing boats, and how different fishery schedules and vessel distribution patterns affect bycatch on the western Mediterranean Sea. Specifically, we used three different approaches: (1) individual GPS trajectories of Scopoli's shearwaters and Vessel Monitoring System (VMS) trajectories of fishing boats to study spatiotemporal dynamics, and establish whether vessel densities determine shearwaters choice between longliners and trawlers; (2) seabird counts on-board longliners to determine the main drivers influencing seabird attendance during longline settings, focusing particularly on the potential influence of trawler activity in the surrounding area; and (3) 13 complete years of bycatch data from one longline vessel to understand whether the rate of bycatch increases on days when trawlers do not operate.

Results

Spatiotemporal interactions. Overall, we tracked 65 shearwaters in two different years, 2010 and 2012, with 4 birds being tracked both years. We obtained trajectories from 90 GPS deployments, 30 trajectories corresponding to 38 foraging trips in 2010 and 60 trajectories corresponding to 145 foraging trips in 2012 (Fig. 1). Birds mainly foraged in the Catalan shelf and the Menorca channel, areas used by both trawlers and longliners (Fig. 1). We obtained 267 interaction events, where a bird followed a vessel, 246 interactions occurred with trawlers and 21 with pelagic longliners. From those, only 86 events corresponded to events in which at least one longliner and one trawler were fishing simultaneously in the same area (Fig. 1). From those interactions, 72 were with trawlers and 14 with longliners. Interactions with longliners mainly happened in the Menorca channel, close to the breeding areas, while interactions with trawlers happened both in the Catalan shelf and in the Menorca channel. We found no direct effect of the number of longliners in the area on the probability of interaction with either a trawler or a longliner. However, the probability of interacting with a longliner increased as the number of trawlers decreased ($P = 0.029$), from nearly 0% when 20 or more trawlers were in the area, to 40% probability of interaction when only 1 trawler was present (Fig. 2).

On-board censuses. We found that the number of attacks to the bait and the number of birds following a vessel was correlated ($r_s = 0.38$, $P = 0.004$). Since sample size for seabird attendance was greater than for bait attacks, we used seabird attendance as a proxy for bycatch risk on subsequent analyses, allowing us to study the ultimate factors that influence it. Analysis of relative importance showed that setting time was the main variable to account for shearwater presence and abundance behind longliners: shearwaters were more likely to occur in greater numbers at twilight (Tables 1 and 2). Bait type and longline type also had an important effect on bird attendance, birds being more likely to interact with longliners when they used mixed baits (fish and cephalopods, instead of only one of both) and when they targeted pelagic species (instead of demersal species) (Tables 1 and 2). The distance to the nearest breeding colony had a relatively high influence on shearwater abundance; the further away from colony areas the less likely it was to detect birds attending longliners (Tables 1 and 2). The number of hooks in each setting had a very low effect on the presence and abundance of birds attending longliners (Tables 1 and 2). The breeding stage of the birds (pre-laying, incubation or chick-rearing) and the meteorological conditions did not affect bird attendance to longliners (Tables 1 and 2). The probability of birds attending longliners increased 4% per each trawler leaving the longliner surroundings, but bird abundance was not affected by the number of trawlers (Tables 1 and 2).

Bycatch data. From 2003 to 2015, we collected all birds hooked in a longline boat from Vilanova i la Geltrú after arrival to the port. During this period, 67 Scopoli's shearwaters became entangled in the fishing gear and died. We found that the number of birds caught differed significantly among the days of the week; it was higher on Sundays and Mondays than on the remaining days ($\chi^2 = 17.63$, $p < 0.001$). In fact, 51% (34 individuals) of the birds were hooked on Sundays, when trawlers do not operate, and 28% (19 individuals) on Mondays, when longline vessels start operating before trawlers after the weekend rest. From Tuesday to Friday, only 14 birds were hooked across the 13 years covered. In addition, we found that the probability of a bycatch event (when one or several birds were hooked) followed the same pattern: events were more likely to occur on Sundays and on Mondays than on the rest of the week days (12 bycatch events occurred on a Sunday, 5 on a Monday and 6 from Tuesday to Friday; $\chi^2 = 4.9$, $p = 0.03$).

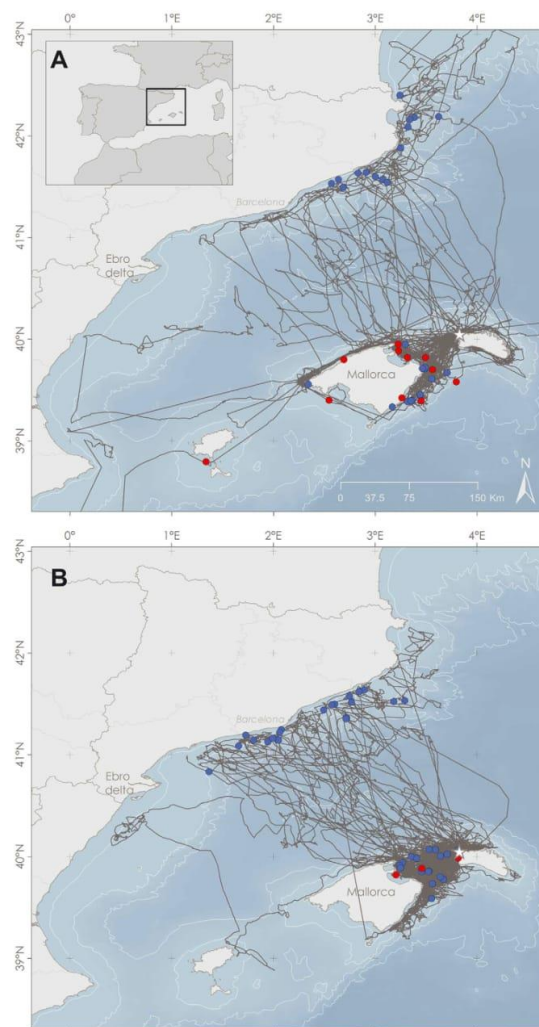


Figure 1. Shearwater GPS tracks (in grey) and concurrent interactions between shearwaters and fishing vessels (dots) inferred from the Vessel Monitoring System (VMS) in 2010 (**A**) and 2012 (**B**). Red dots correspond to interaction with longliners and blue dots to interactions with trawlers. Maps were generated with ArcGis version 10.3 (URL: <https://www.arcgis.com>).

Discussion

The concurrent analysis of GPS data from seabirds and vessels showed the importance of the spatiotemporal distribution of operating trawlers in determining the probability of seabirds interacting with longliners: the higher the density of trawlers, the less likely birds interacted with a longliner. On-board censuses of seabird attendance to longliners corroborated this result, showing that attendance, which can be taken as a proxy of bycatch risk, increased when trawlers were not operating in the longliner proximity. Finally, dead birds collected by fishermen showed that seabird catches were significantly and substantially greater on days when trawlers did not operate. Hence, results from these three different approaches point towards the same direction: seabird bycatch in longliners significantly increases when trawlers operate in low densities or do not operate.

In this regard, the reform of the CFP, which intends to substantially reduce or even eliminate fishery discards, might dramatically increase seabird bycatch risk, at least in the short term, by forcing seabirds to intensify their foraging efforts, including the search of an “easy meal”, i.e. switching from trawler discards to longline baits. Our results show that, at present, trawlers are acting as a buffer of the seabird interactions with longliners, such that the probability of shearwaters interacting with a longliner decreases from 40% when only one trawler is present in the area to almost 0% when >20 trawlers are present (Fig. 2). The same pattern applies to the on-board

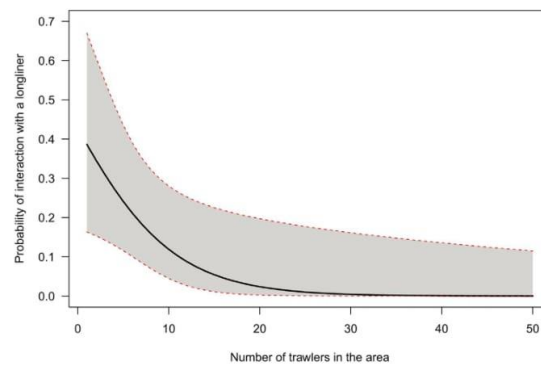


Figure 2. Probability of interaction between shearwaters and longliners as a function of the number of trawlers in the area. Black line represents the predicted values from the model and the grey area represents the 95% confidence intervals.

Variable	Value	Std. Error	RI
Presence - Zero part (Intercept)	−0.84	1.01	
Bait: Fish	1.75	1.11	0.93
Bait: Mixed	2.73	1.38	
LT: Pelagic	1.96	1.31	0.59
Time: Daytime	−1.39	0.79	0.90
Trawlers	−0.18	0.24	0.53
SDC	−0.27	0.45	0.42
Period: Incubation	0.11	0.47	0.35
Period: Prelying	−0.41	0.74	
Wind: Windy	−0.07	0.34	0.24
SHS	0.02	0.16	0.24
Abundance - Count part (Intercept)	2.48	0.47	
LT: Pelagic	0.15	0.40	0.31
Time: Daytime	−0.53	0.46	0.76
Trawlers	−0.01	0.09	0.22
SDC	−0.15	0.19	0.55
Period: Incubation	0.01	0.07	0.05
Period: Prelying	0.01	0.11	
Wind: Windy	−0.03	0.16	0.20
SHS	−0.08	0.14	0.39

Table 1. Coefficient, standard errors and relative importance (RI) of each variable for shearwaters attendance to longliners. See Table 2 for detailed explanation of the variables.

observations and bycatch rates. For each trawler leaving the surroundings of a longliner, the probability of seabirds following the longliner increases by 4%; and the rate of bycatch experiences a tenfold increase when trawlers do not operate. The reduction of discards is therefore likely to result in a substantial increase in bycatch rates of Scopoli's shearwaters, to a level that could be completely unsustainable for some western Mediterranean populations²⁹. Moreover, our results can reasonably be extrapolated to other seabirds species targeting trawler discards in the Mediterranean which are also known to be caught in longlines, such as the Audouin's gull and the Yelkouan and Balearic shearwaters²³. The latter is of particular concern given its sensitive conservation status, since bycatch appears to account for almost half the adult mortality estimated for the species⁸.

Given the concern of bycatch for many Mediterranean seabirds, including the four endemic species, it is urgent to take into account this multi-fisheries interaction when designing fishing regulations, in order to minimise its potentially detrimental effect. As a first approach, our results suggest that precluding longline vessels (both demersal and pelagic) to set their lines when trawlers are not operating might substantially contribute to this aim. However, given that (trawling) discards will anyway be reduced in the short run, our results also call for an immediate enforcement of effective mitigation measures in longliners to reduce seabird bycatch. Since our results also point out that the strongest influence on seabird attendance to longliners was the time of setting, operational measures regulating the setting timing should contribute to minimise the problems. Indeed, seabirds were more prone to interact with longliners during sunset and sunrise. These results have also been observed in

Variable	Abbreviation	Type	Description
Breeding period	Period	Categorical	Pre-laying (March – 1st week June), incubation (until mid-July) or chick-rearing (until October)
Distance to colony	SDC	Continuous	Distance from the nearest colony (scaled km)
Wind	Wind	Categorical	Windy or still
Bait composition	Bait	Categorical	Fish, cephalopods or mixed (fish + cephalopods)
Setting time	Time	Categorical	1 h \pm of the twilight or rest of the daytime
Longline type	LT	Categorical	Demersal or pelagic
Hooks setting	SHS	Continuous	Number of hooks setting (scaled)
Number of trawlers	Trawlers	Continuous	Number of trawler within 6 km from the longliner
Longliner ID		Random	Boat identifier

Table 2. Explanatory variables used in the analyses of Scopoli's shearwaters interaction with longliners.

other studies which showed that many diurnal seabirds have activity peaks at dawn and dusk^{19,27,30}. Since most seabirds affected by longliners in the Mediterranean are basically diurnal, a promising mitigation measure to be applied in this region would be night setting, as previously suggested in other studies^{19,30–34}. This measure could be easily implemented at low economic costs, and compliance could be monitored and enforced to some extent through the control of fishing schedules of longliners by harbour authorities, as it is currently done for other types of fisheries. However, fishermen could be reluctant because it would require a rearrangement of their schedules and it could also limit the number of setting operations, particularly during the relatively short summer nights. Therefore, further work is needed to assess the efficacy and viability of this measure as well as of other mitigation measures that have proven successful in other regions. Among them, the use of tori-lines, the increase of sinking rates of the line through configuration changes, or a combination of the above, might also contribute to minimise seabird bycatch in Mediterranean longliners^{31,34–37}.

In conclusion, our study highlights the importance of combining various sources of information to achieve robust and complementary results on the complex effects of fishing activities on seabird bycatch. In particular, three different approaches indicated that the risk of a seabird to be captured in longlines increases dramatically when trawlers are not present in the area where longliners operate. That is, when trawlers stop providing discards seabirds may switch from trawlers to longliners, and therefore the landing obligation being implemented by the CFP must be carefully monitored and counterbalanced with the urgent implementation of mitigation measures. In a more general sense, our results point out that to determine the best management practices of the different fishing fleets, we need to study unexpected impacts rising from the interactions among different types of fisheries. Therefore, impacts of changes on the discard availability must be carefully evaluated and monitored across the different fleets to avoid catastrophic effects on seabird populations as well as on other components of the marine ecosystem.

Methods

Spatiotemporal interactions. To establish the spatiotemporal interaction between tracked birds and fisheries, we obtained data from two main sources: GPS devices for tracking shearwaters and the VMS for tracking vessels. GPS-tracking of Scopoli's shearwaters was conducted in Cala Morell (Menorca, Balearic Islands, Spain; 40°3'N, 3°52'E), in two different years. In 2010, birds were tracked during the incubation period, from the 18th June to the 8th July; and in 2012 during the chick rearing period, from the 25th July to the 20th September. This area holds the largest Scopoli's shearwater colony of the Balearic Islands, tentatively estimated in 1,000–6,000 pairs^{38,39}. Scopoli's shearwaters were captured at night by hand or using looped poles on the nest, when they flew back to the colony to feed their offspring or for incubation shifts. We used GPS loggers (Perthold Engineering LLC, weighing 20 g⁴⁰) sealed to be waterproof and programmed to record bird position each 2.5 or 5 minutes. Loggers were attached to the back of the birds using Tesa® tape⁴¹. At deployment, birds were ringed, sexed (through biometric measures) and weighed. In an attempt to minimise adverse effects on the birds, total mass of the device did not exceed 3% of the birds body mass⁴². At recovery, GPS devices were detached and birds were weighed. On average, we found a 25 g decrease on bird weight after tag retrieval (Paired t-test, $t = 4.4138$, $df = 83$, $p < 0.001$), that we do not expect to have relevant effects on the foraging behaviour of the birds⁴³. In 2010, we deployed 30 GPS tags in 25 individuals, whereas in 2012, 79 GPS loggers were deployed on 56 individuals. In order to minimise the possible impact of tagging birds on their breeding success, only one adult bird per nest was tagged. Birds carried loggers from 3 to 17 days before retrieval, recording from 1 to 9 foraging trips. These protocols were approved by *Servei de Protecció d'Espècies*, from the Balearic Islands Government. The methods were carried out in accordance with the relevant guidelines and bird handling and tagging protocols.

The Vessel Monitoring System (VMS) is a satellite-based monitoring system, implemented by the European Union, that provides data on the location, course and speed of fishing vessels over 12 m long⁴⁴. In the Spanish Mediterranean, 90% of trawlers and 60% of pelagic longliners use this localization system, although only a few demersal longliners and artisanal (polyvalent) vessels use it⁴⁵. The default frequency of VMS locations is one fix every two hours. Consequently, the spatiotemporal combination of VMS and GPS data was obscured by the uncertainty about the position of each vessel in the two hour gap between two consecutive locations. Taking into account this limitation, to cover the potential interactions between birds and vessels throughout the entire vessel trip, we identified all bird locations within a ± 1 h interval and within a 5 km buffer from a vessel location (as maximum speeds of vessels are around 5 km/h). Next, we applied a second filter selecting bird trajectories where

the bird bearing diverged in less than $\pm 30^\circ$ from the vessel bearing (estimated from consecutive locations). This bird bearing was considered the mean bearing for all locations inside each buffer of spatiotemporal coincidence. We chose $\pm 30^\circ$ because after some trials this angle emerged as the most biologically meaningful figure. For each bird-vessel interaction location, we assessed the number of vessels within a ± 1 h interval and a 30 km radius. We chose 30 km as it has been shown that some procellariiforms can detect food resources up to 30 km away^{46,47}. We focused our research in two types of fisheries, trawling and pelagic longlining, since shearwaters tend to associate with them in search of food. Demersal longliners, including artisanal (polyvalent) vessels were excluded from this analysis, despite also attracting (and catching) seabirds, since most of them are too small to carry the VMS system (see above). Moreover, we only selected the interaction events where at least one trawler and one longliner were present in the area to control for the fisheries different regime and ensure that when birds interacted with a trawler they had also the option to interact with a longliner and vice versa.

We used a generalized lineal mixed model that included year as a random effect, number of trawlers and number of longliners as fixed effects and a binomial response, either the bird interacted with a longliner or it interacted with a trawler. We calculated the AIC values of all candidate models and selected the model with the lowest AIC value as the best model for explaining bird interaction with fisheries.

On-board censuses. At-sea surveys were carried out during 3 consecutive years (2011–2013) covering the main fishing grounds of longline fisheries in the Catalan shelf and Balearic Islands, during the period where the species was present in the Mediterranean (March–October). The counts were conducted during 102 longline settings from 20 small-scale vessels operating in the NW Mediterranean (16 demersal and 4 pelagic longliners). Here we only considered the maximum number of Scopoli's shearwaters following the vessels at the end of each 10-min counts in each setting operation, as well as the number of attacks to the bait and the number of birds incidentally captured. The number of birds hooked (26 birds in nine events) was too low to perform reliable statistical analyses. Fishing habits, detailed description of fishing gear used and meteorological data were noted in each fishing trip (Table 2). The number of trawlers in a 6 km radius around the longliner was also recorded in each 10-min survey.

Bycatch events are relatively rare and patchy, thus being difficult to monitor through low-effort observer programmes. We evaluated seabird attendance to longliners as a proxy of seabird attacks, which are more closely related to seabird bycatch and therefore mortality. Generalized lineal mixed models (GLMMs) were used to identify the main factors influencing Scopoli's shearwater interaction with longliners. Abundance data is characterized by having a high proportion of zero values and a skewed distribution of non-zero positive values caused by large counts of individuals (flocking behaviour)⁴⁸. Hurdle models are a suitable method for modelling this type of distributions, which is characterized by treating the data in two parts: (1) presence/absence of the species (Zero part) and (2) the abundance when the species is present (Count part)^{48–51}. We analysed the relationship between the number and presence of Scopoli's shearwaters and temporal, spatial and operational variables (Table 2). Trawler presence in the surrounding area was also considered to assess their influence on seabird attraction to longliners. Longliner identity was used as a random effect. We used “glmmadmb” function from the “glmmADMB” R package (R version 3.1.2). Zero hurdle part was modelled with the assumption of a binomial error structure (logit link function), while in the Count part a truncated version of the Negative Binomial distribution was considered (log link function). We checked collinearity between predictors and removed redundant ones. Then, we used the variance inflation factor (VIF) to verify the independence of each variable on the estimate of the regression coefficients of the model⁵².

Relative importance analysis was carried out with the model-averaging approach. This approach is useful when there is a large uncertainty about a set of models⁵³. In this way, we obtained model-averaged parameter estimates that were directly comparable to each other⁵³. We estimated the parameters from the set of all models for which the sum of Akaike weights reached >0.95 .

Bycatch records. From 2003 to 2015 fishermen from a single demersal longline vessel fishing off the Catalan coast recorded and handed over Scopoli's shearwater carcasses accidentally caught in their longline. To establish temporal bycatch patterns, we analysed whether the number of birds hooked and the number of capture events differed among the days of the week by using a Pearson's Chi-squared Test for Count Data with Bonferroni correction.

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Author Contributions

A.S.R. collected and analysed the data and wrote the paper; V.C. collected and analysed data and made comments in the manuscript; J.M.R.G. and S.G. provided advice in analytic and ecological issues and logistic support throughout all the stages of the study and discussed drafts of the manuscript; J.B., B.R. and J.M.A. collected the data and made comments on the manuscript and J.G.S. conceived the study and wrote the paper.

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